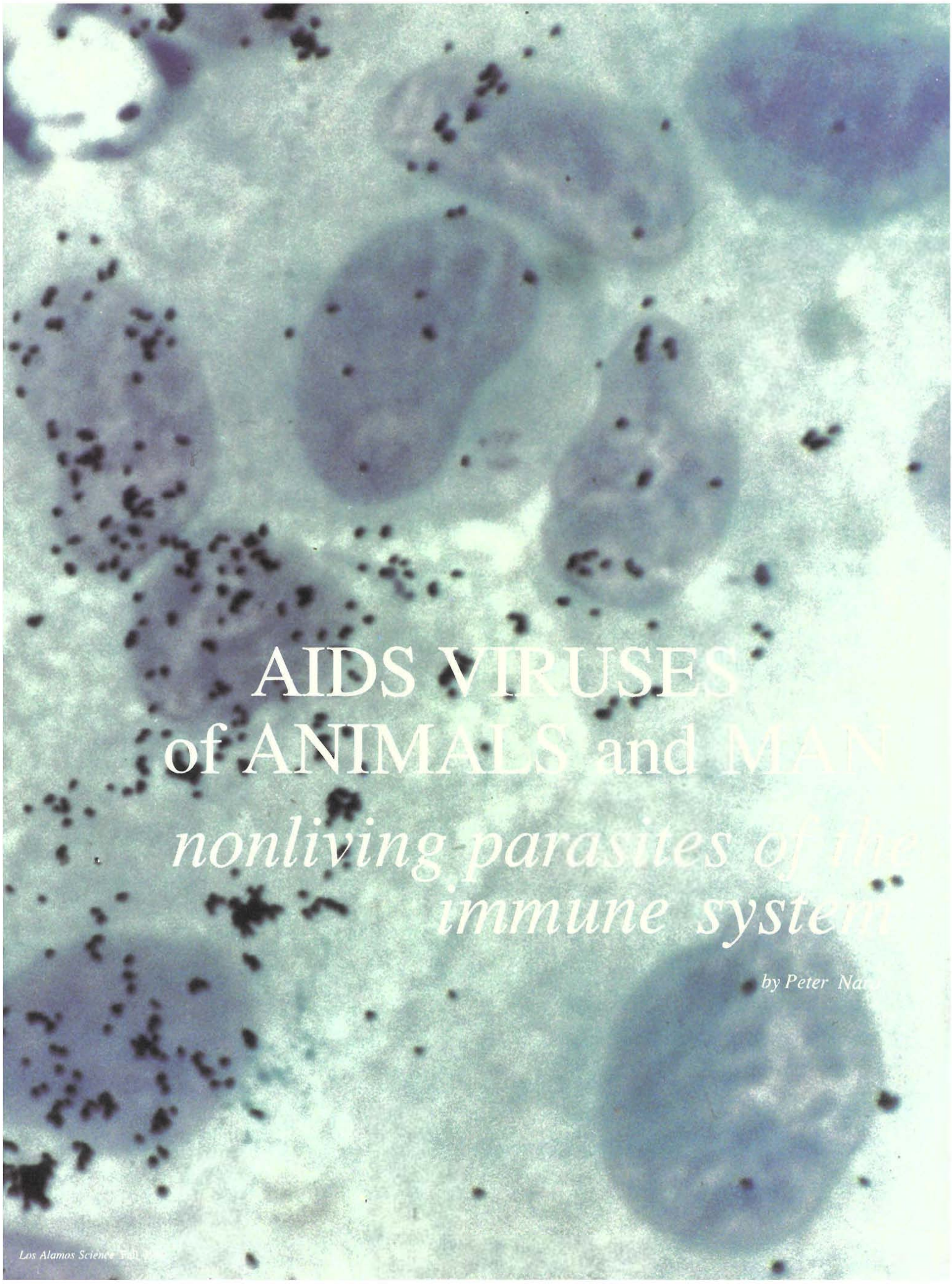


AIDS VIRUSES of ANIMALS and MAN

nonliving parasites of the immune system

by Peter Nar

From one of the earliest known descriptions of a viral disease, namely, the report in 1904 of a disease affecting horses called swamp fever, to the sudden appearance of a wasting disease of sheep, which reached epidemic proportions in Iceland in the 1940s, the *lentiviruses* remained known only among a small group of virologists as a curiously slow infectious agent and were totally unappreciated as a potential source for a global human viral pandemic. Then, in the late 1970s and early 1980s, subtle patterns of a new clinical disease gave way to the parallel epidemics of AIDS in captive primates and humans. Characterized by seemingly unrelated opportunistic infections, the lentiviruses thrust themselves into the scientific, genetic, moral, and cultural fabric of mankind throughout the world.

HIV (human immunodeficiency virus), the cause of AIDS, is a member of the lentiviruses, a subfamily of a larger family called *retroviruses*. That large family is well known for containing viruses that cause cancer in humans and other animals. Although lentiviruses do not cause cancer, they do present a formidable challenge to the host. First, lentiviruses integrate themselves into their host's genetic blueprint. Second, they contain numerous regulatory genes that allow them to control their rate of replication in both dividing and nondividing cells. Third, and most important, they have evolved, interacted, and survived completely within the cells of the host's immune system—the only viruses described to date that spend their entirety in such cells.

In this article we intend to retrieve from anonymity the lentiviruses associated with animals other than humans and focus attention on their various strategies for survival. We will explain how the AIDS virus and other lentiviruses outsmart the host's immune system and show why traditional approaches to vaccine development will most likely fail against this type of virus. Finally, we will turn to models of host adaptation, in particular, the African green monkey and the chimpanzee, as a probable source of inspiration for understanding and, therefore, developing a successful strategy against AIDS and AIDS-like diseases.

Retroviral Life Cycle and Family History

All viruses are parasitic in nature. They require a host to replicate but unlike parasites, which are living organisms, viruses are functionally nonliving. A virus is best described as an infectious chemical made up of an outer envelope or protein coat that encapsulates the viral genome, the genetic blueprint for constructing more viruses. What they lack are the protein-synthesis and energy-generating capabilities required to manufacture progeny. They infect the host cell by binding to and fusing with the cell's membrane and then depositing the viral genes within the cell where they are free to be read by and interact with the host's manufacturing machinery (Fig. 1).

The "retro"viruses are so-called because at the beginning of their life cycle they reverse the usual flow of genetic information. In all living organisms and in many other viruses, genetic information is stored as deoxyribonucleic acid, or DNA, and later transcribed into ribonucleic acid, or RNA, which serves as a template for protein synthesis. By contrast, retroviruses store their genetic information as RNA and also contain the unique enzyme, reverse transcriptase, which catalyzes the "reverse" transcription of the RNA genome into a DNA copy. The resulting proviral DNA is oftentimes perceived by the host cell as its own and is integrated into its DNA where the provirus can remain dormant or latent for weeks, months, or even years without being expressed. In fact, some retroviruses (for example, those of chickens and mice) have assured their persistent association by integrating into the germ cells of the host. As integrated viruses (so called proviruses), they are transmitted vertically to the next generation without an infectious cycle. There are no known methods of eliminating such retroviruses.

The retrovirus family, evolutionarily speaking, is quite old. It contains three subfamilies: the oncoviruses, the spumaviruses, and the subfamily of most interest to us, the lentiviruses (Table 1). The oncoviruses, or cancer-causing viruses, are found to be transmitted both by host-to-host contact and as integrated viruses in germ cells. When integrated into the host's DNA, oncoviruses efficiently "transform" the host cells into cells that have a tumor-

STRUCTURE AND LIFE CYCLE OF HIV

Fig. 1. The structure (a) and life cycle (b) of HIV. The cycle starts with the binding of the viral envelope protein *gp*120 to a CD4 receptor on the surface of the target cell, the fusing of the viral and cellular lipid bilayers, and the entry of the viral core, containing the RNA genome and the enzyme reverse transcriptase, into the cell's interior. The cycle ends with the production of new viral genomes and viral proteins and the assembly of viral cores and budding of new virus particles. Step 4, reverse transcription of the viral genome into proviral DNA, and step 5, integration of proviral DNA into the host cell's genome, are unique to retroviruses. In some cases, after step 4, the DNA will spontaneously close on itself and this circular DNA will remain in the cytoplasm as episomal DNA. Also shown is the possibility that steps 4-6 will be bypassed and the positive strand of genomic RNA will serve directly as a template for protein synthesis, that is, it will be translated directly into viral proteins by ribosomes within the cell.

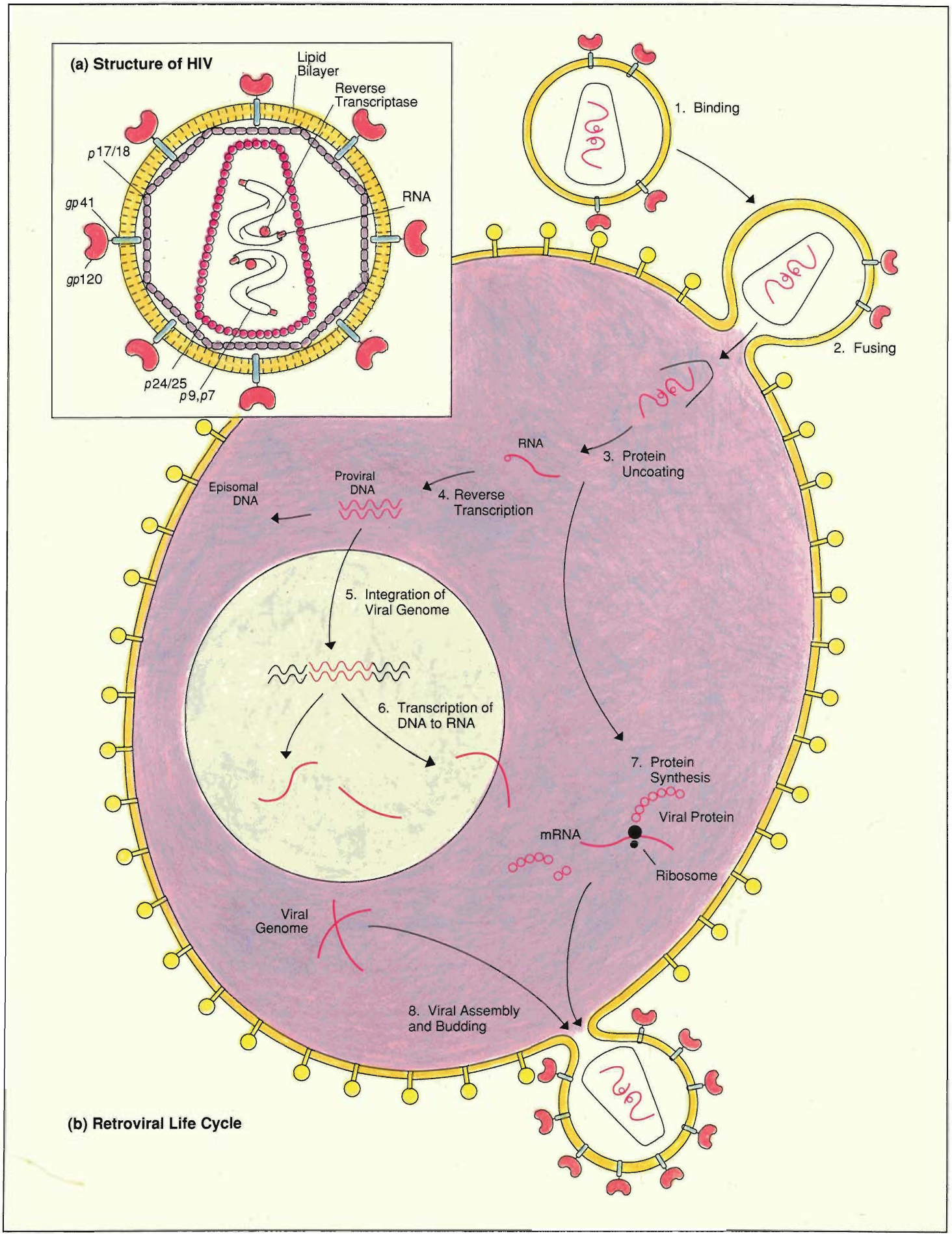

(a) Structure of HIV
Lipid Bilayer
Reverse Transcriptase
p17/18
gp41
gp120
RNA
p24/25
p9,p7
1. Binding
2. Fusing
RNA
3. Protein Uncoating
Episomal DNA
Proviral DNA
4. Reverse Transcription
5. Integration of Viral Genome
6. Transcription of DNA to RNA
7. Protein Synthesis
Viral Protein
mRNA
Ribosome
Viral Genome
8. Viral Assembly and Budding
(b) Retroviral Life Cycle

Table 1

Subfamilies of the Retroviruses

Oncoviruses: Retroviruses that are transforming (that is, they create a tumor-producing potential in infected cells) or closely related nontransforming viruses.

- Murine intercisternal A (Type A)
- Mouse mammary tumor virus (Type B)
- Avian leukosis virus (Type C, avian subgroup)
- Moloney murine leukemia virus (Type C, mammalian subgroup)
- Mason Pfizer monkey virus (Type D)
- Bovine leukemia virus (BLV/HTLV)
- Human T-cell lymphotropic virus (BLV/HTLV, Types I and II)
- Simian T-cell lymphotropic virus (BLV/HTLV, Type I)

Lentiviruses: Pathogenic slow viruses that cause persistent multiorgan disorders and are exogenous, that is, they do not integrate themselves into the host's germ cells.

- Visna maedi virus
- Caprine arthritis encephalitis virus
- Equine infectious anemia virus
- Feline T-lymphotropic virus
- Bovine immunodeficiency-like virus
- Simian immunodeficiency virus (SIV)
- Human immunodeficiency virus (HIV, Types I and II)

Spumaviruses: Foamy viruses that cause persistent infections without clinical disease.

- Simian foamy viruses (9 serotypes)
- Bovine syncytial virus
- Feline syncytial virus
- Hamster foamy virus
- Human foamy virus

producing potential. Oncoviruses have been found in either complete or incomplete form within various normal tissues and developing embryos of many species of animals including humans. Their presence in the host through evolutionary spans of time may have been responsible for the shuffling of critical genetic elements between cells during various embryological and differentiative processes, which subsequently led to Darwinian selection.

The lentiviruses and spumaviruses (spuma for foamy) have a somewhat different relationship with the host. These viruses do not integrate into the host's germ cell lines and do not cause cancer. In vivo both produce lifelong infection of the host cells but may not kill the infected cells. In vitro they infect and kill host cells through massive viral replication and tearing of the cell membranes as they bud from the cell surface. The genomes of spumaviruses and lentiviruses are more closely related to each other than to those of the oncoviruses. However, of the two, only the lentiviruses have been identified as causes of human and animal diseases.

All retroviruses are rapidly changing because reverse transcription of their RNA genomes often produces mistakes in the DNA copies. In oncoviruses such "mistakes" sometimes create defective viruses, that is, pieces of viral DNA that are incorporated into the host genome but cannot replicate, although their presence may promote the growth of tumors. Under the proper conditions certain other helper viruses "rescue" these defective viral pieces by also incorporating themselves into the host genome and creating new genetic combinations that can replicate. The rescued oncoviruses usually have different physical, biological, and tumor-inducing properties than the original virus. Another type of genetic recombination has also been found in experiments with mice. Genes that specify the envelopes for two different retroviruses or retroviral strains are exchanged. The exchange confers on the viruses the ability to infect new cell types or cells of another species. Recombination of envelope genes can also enable the virus to escape both specific and nonspecific antiviral substances found in the host. Many of these types of genetic recombinations have recently been found to occur in human cells infected by HIV. As we will discuss below, spontaneous mutations, immune selection, and genetic recombination in HIV presents one of the major blocks to developing a traditional vaccine against AIDS.

The Immune System—Host for the Lentiviruses

The central problem in the evolution of multicellular organisms is the recognition of foreign from self. The body, a multicellular organism, may be thought of as an ecosystem containing numerous niches that can be occupied by organisms uniquely adapted to the prevailing environment. One such niche in this ecosystem is the cell, which, like other living members of a natural ecosystem,

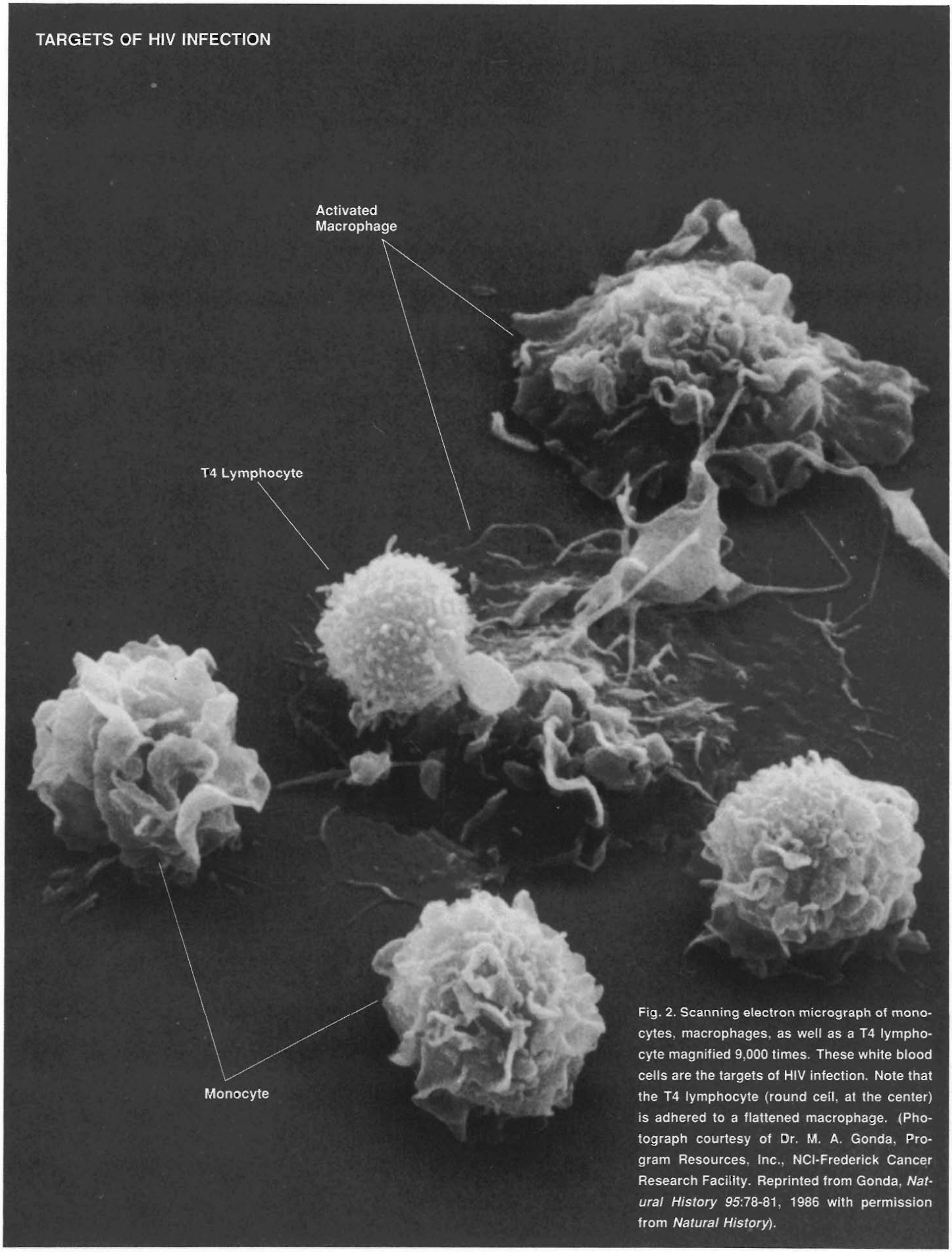
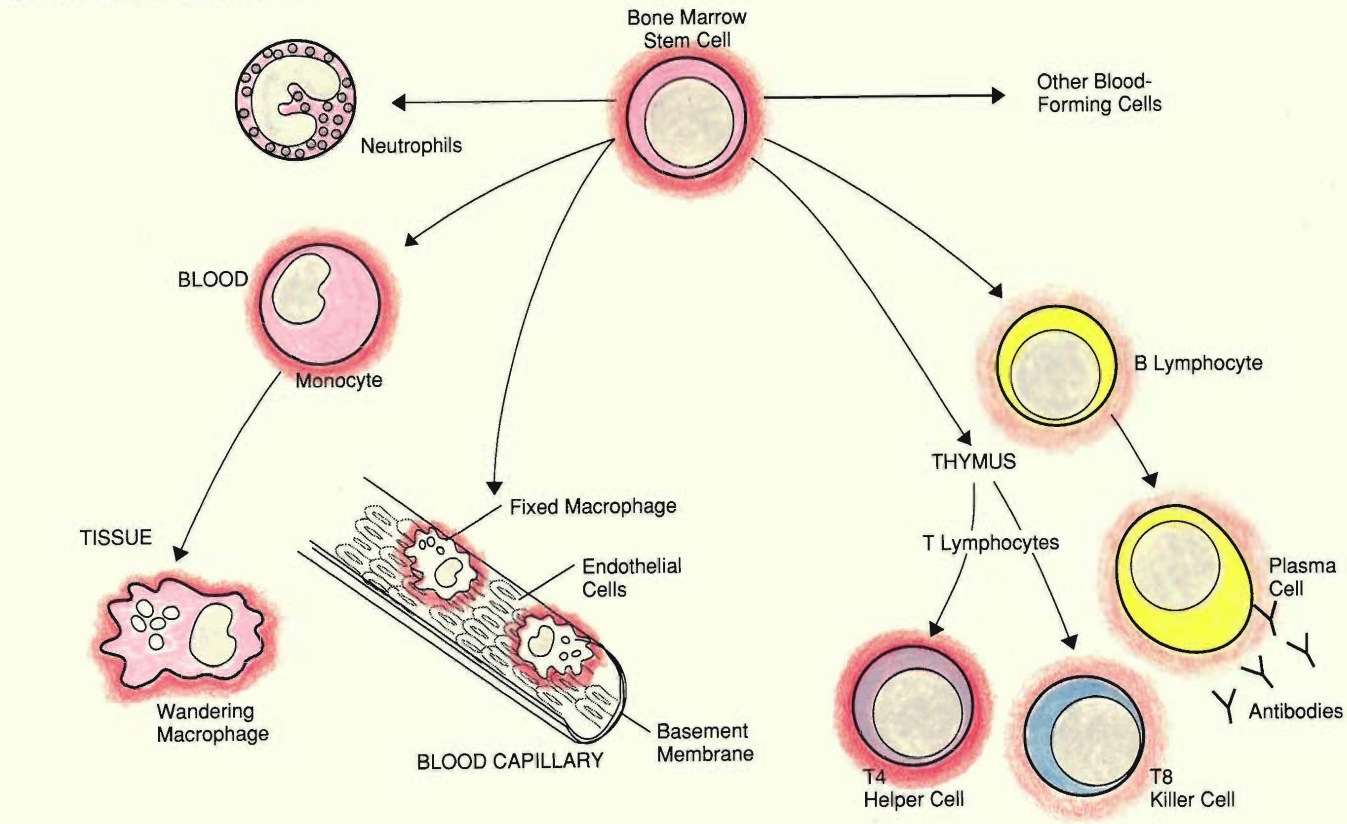
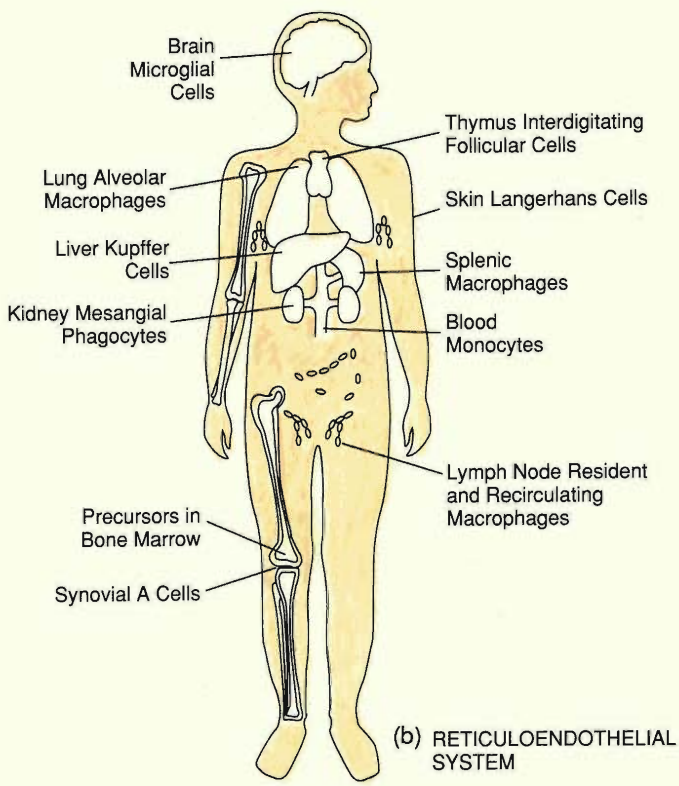
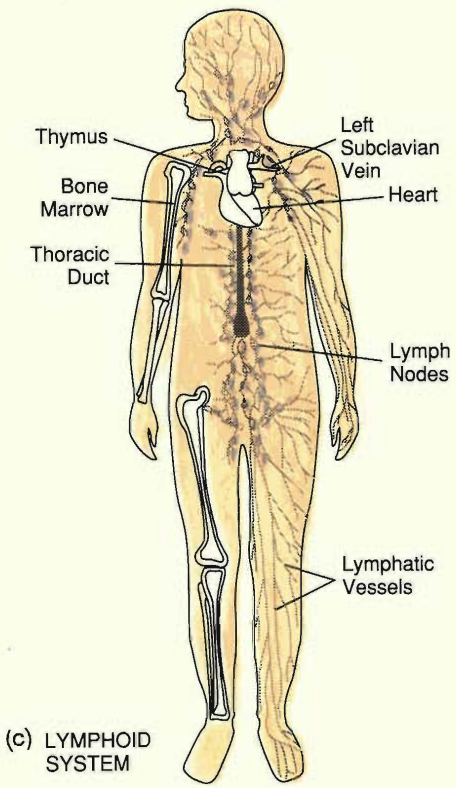


Fig. 2. Scanning electron micrograph of monocytes, macrophages, as well as a T4 lymphocyte magnified 9,000 times. These white blood cells are the targets of HIV infection. Note that the T4 lymphocyte (round cell, at the center) is adhered to a flattened macrophage. (Photograph courtesy of Dr. M. A. Gonda, Program Resources, Inc., NCI-Frederick Cancer Research Facility. Reprinted from Gonda, *Natural History 95*:78-81, 1986 with permission from *Natural History*).

WHITE CELLS OF THE IMMUNE SYSTEM







THE IMMUNE SYSTEM

Fig. 3. (a) White cells of the immune system. The cells surrounded by deep red halos are the primary targets of HIV infection; those surrounded by pale red halos are less frequent targets. Among the primary targets are monocytes and macrophages (upper left), part of a functional system of scavenging cells (phagocytes) and antigen-presenting cells called the reticuloendothelial system (b). Whereas monocytes circulate in the blood, macrophages are strategically located in various organs of the body and in the lymph nodes. (Note that the monocytes and macrophages have kidney-shaped nuclei.) Another line of immune defense is provided by the lymphocytes (upper right), an adaptive system that acts against specific foreign antigens (proteins). T lymphocytes mature, differentiate and acquire their antigen-specificity in the thymus. In the presence of specific foreign antigens, the T4 helper lymphocytes direct the activities of other immune cells by sending out chemical messages and the T8 killer lymphocytes send out cytotoxins, which kill the foreign cells. B lymphocytes, in the presence of specific foreign antigens, mature into plasma cells and manufacture Y-shaped antigen-specific protein molecules called antibodies, which bind to the foreign antigens. Most white cells of the immune sytem flow through the bloodstream, and at specific sites in capillary walls, they exit to the lymphatic system (c), a network of tiny vessels permeating the body whose walls are only one-cell thick. These tiny vessels act as conduits for white cells and collect all the extracellular lymphatic fluids in the body. These cells and fluids are then recirculated back to the bloodstream through vessels that merge into even larger lymphatic ducts, like the streams in a watershed, and eventually converge into the large thoracic duct, which empties into a large vein at the base of the neck called the left subclavian vein. The flow of white cells through the lymphatics and bloodstream provides continual immune surveillance of the entire body.

can be invaded by disease-producing organisms. An additional problem for the body's ecosystem is the spontaneous generation of mutant cells, that is, tumor cells that could threaten its own survival. Thus, depending on the size of the multicellular organism, a unique set of cells had to evolve to assure that no members of the organism's ecosystem be parasitized by intracellular pathogens or altered in a way that would damage their critical day-to-day functions. In a large number of animals, including man, that set is composed of two special types of white cells (Fig. 2): the *monocytes* and *macrophages*, and the *T lymphocytes* (the T stands for thymus-derived). Although evolutionarily they are among the oldest cells of the immune system and are well-adapted to perform their functions, these cells have provided the perfect niche for certain nonliving parasites—namely, the lentiviruses! To understand the impact of lentiviral infections, we will first outline the genesis and normal functioning of monocytes, macrophages, and T lymphocytes.

The immune system is a complicated network of white cells and their chemical products (Fig. 3), which interact synergistically to eliminate foreign invaders, abnormal cells, and toxic cell products. White cells generally originate from stem cells found in foetal liver and bone marrow. As they mature, they differentiate into many cell types with separate or overlapping functions (Fig. 3a). Most white cells in the blood are short-lived scavenger cells (neutrophils) that engulf and digest foreign microbes and die. The pus seen in bacterial infections are primarily these dead scavenger cells.

The monocytes and macrophages are also scavenger cells, but they may live months or years. They are particularly good at detecting, engulfing, and digesting tumor or virally infected cells. Some monocytes circulate in the bloodstream and later in their life receive immune signals from the lymphocytes that cause the monocytes to migrate into tissues and transform into tissue-specific macrophages. There they either wander freely through the connective tissue in organs or attach to the basement membranes of the tiny capillaries in those organs. Other monocytes differentiate directly into tissue-specific macrophages. Macrophages are concentrated and strategically located in the liver, lungs, and lymph nodes—organs that receive blood from parts of the body exposed to the outside world, such as the gastrointestinal or respiratory tract. Should the invading pathogen escape this early level of immune defense, other macrophages located in the spleen, kidney, joints, and brain provide a second level of defense. Together, monocytes and macrophages form what is known as the *reticuloendothelial system* (Fig. 3b), one major line of defense in the immune system.

Another major line of defense in the immune system is the *lymphoid system*, a set of glands, organs, and cells (Fig. 3c). The lymph nodes, which are distributed throughout the body, serve as way stations, storage facilities, and manufacturing and shipping sites for specific cells of the immune system, including the T and B *lymphocytes* (literally meaning cells of the lymph). In the process of maturing, the lymphocytes differentiate into hundreds of thousands of lymphocyte subgroups, each very small and each designed to recognize and mount a defense against a specific foreign protein, or *antigen*. But how does each antigen-specific subgroup prepare its attack when its target antigen enters the body?

Macrophages entering the lymph nodes or interacting with lymphocytes in tissues have the job of "presenting" foreign antigen to the appropriate lymphocyte subgoup and thereby activating it. More specifically, when macrophages engulf and digest foreign microbes or

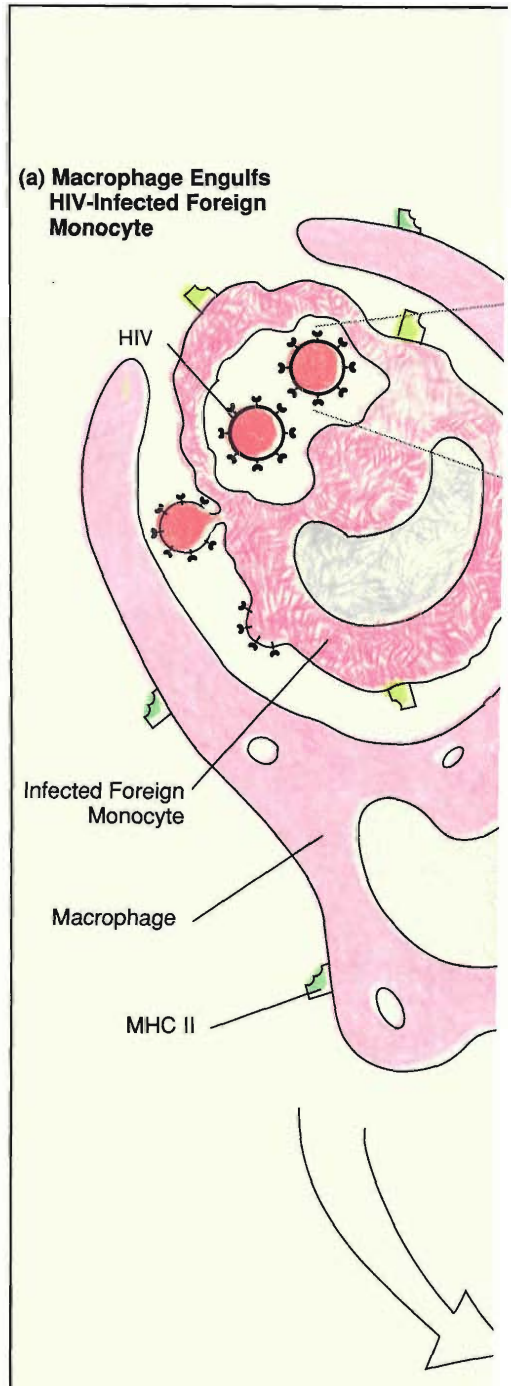
infected cells, they incorporate into their surface membranes the proteins of the foreign invaders (Fig. 4). The foreign antigens are inserted on the macrophage surface next to other normal receptors, called *MHC antigens* (for antigens of the major histocompatibility complex). The MHC antigens are part of the associative recognition network of surface receptors that enable the macrophages and lymphocytes to recognize each other as parts of the self and to receive appropriate instructions from each other. When a foreign antigen is present in the macrophage surface, only those lymphocytes that recognize or bind to both an MHC receptor and the specific foreign antigen are activated. Figure 4 illustrates this dual recognition by lymphocytes. In this case, we have chosen to show how a special type of lymphocyte called a *T4 helper cell* recognizes both an MHC II receptor and the antigen *gp120*, one of the envelope proteins of HIV. Although this example is particularly relevant for our story, it also illustrates the normal phenomenon of recognition between antigen-presenting macrophages and lymphocytes.

Figure 5 goes on to illustrate the many immune responses induced by the dual recognition between a T4 helper cell and an antigen-presenting macrophage. Contact between the T4-cell receptors and the MHC and foreign antigens of the macrophage stimulates the T4 lymphocyte to send out chemical instructions to other immune cells. The chemical instructions induce a variety of effects: they activate monocytes and macrophages and thereby enhance their ability to engulf and destroy the invading pathogen; they stimulate cytotoxic lymphocytes, called *T8 killer cells*, to proliferate and kill cells that display the foreign antigens on their surfaces; and they stimulate B lymphocytes (the B stands for bursal or bone marrow-derived) to proliferate and produce antigen-specific antibodies capable of binding to the foreign antigen. The antibodies produced by B lymphocytes help the macrophages and other cells to carry out their function either as scavenger cells or as killer cells.

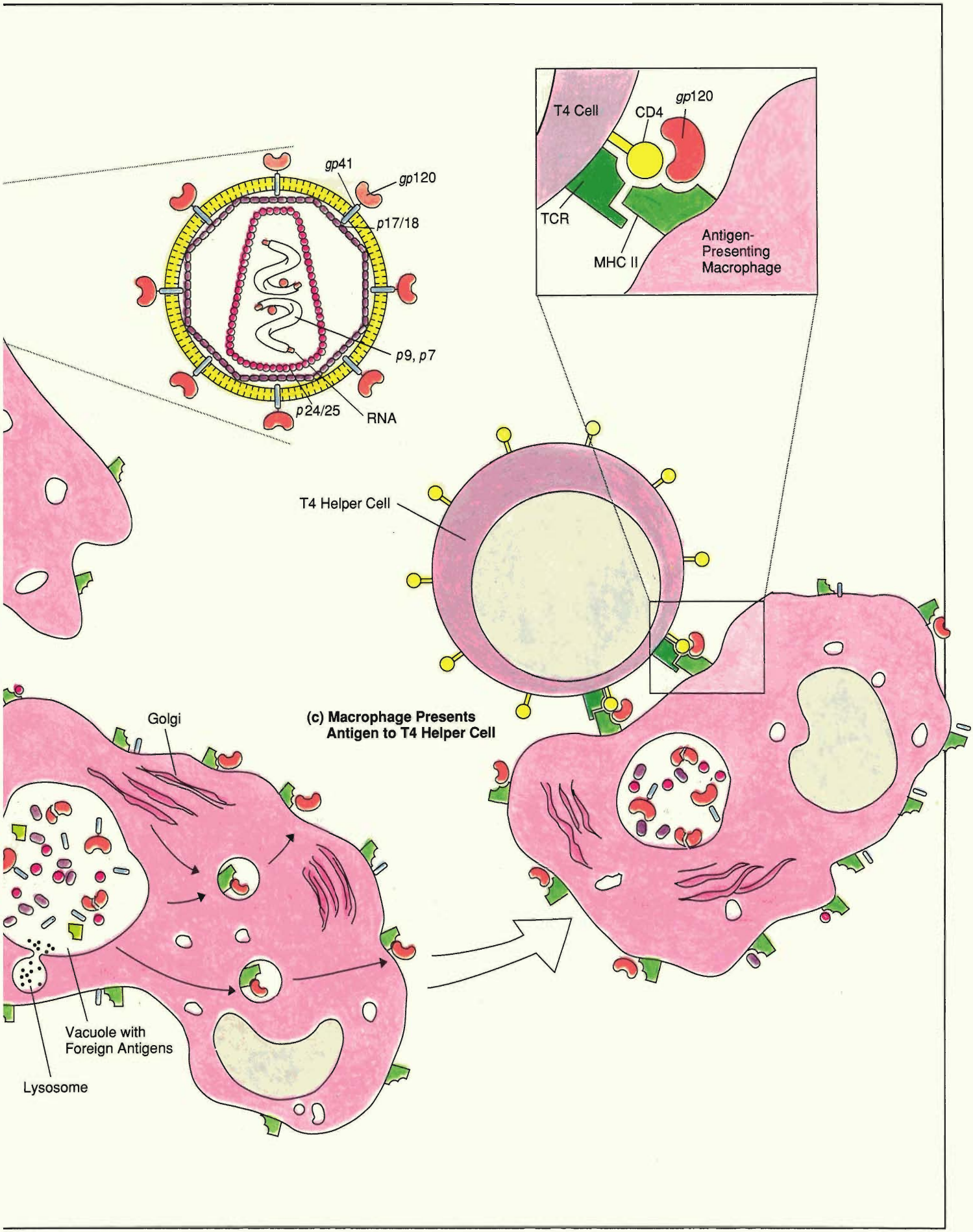
In addition to all this complex activity against a foreign invader, the body must keep the immune response from getting out of hand. Control is accomplished by conveniently activating other T lymphocytes, called *T8 suppressor cells*, which produce chemical messages capable of slowing or stopping the immune reactions. Thus, when the macrophages and lymphocytes interact, they mount

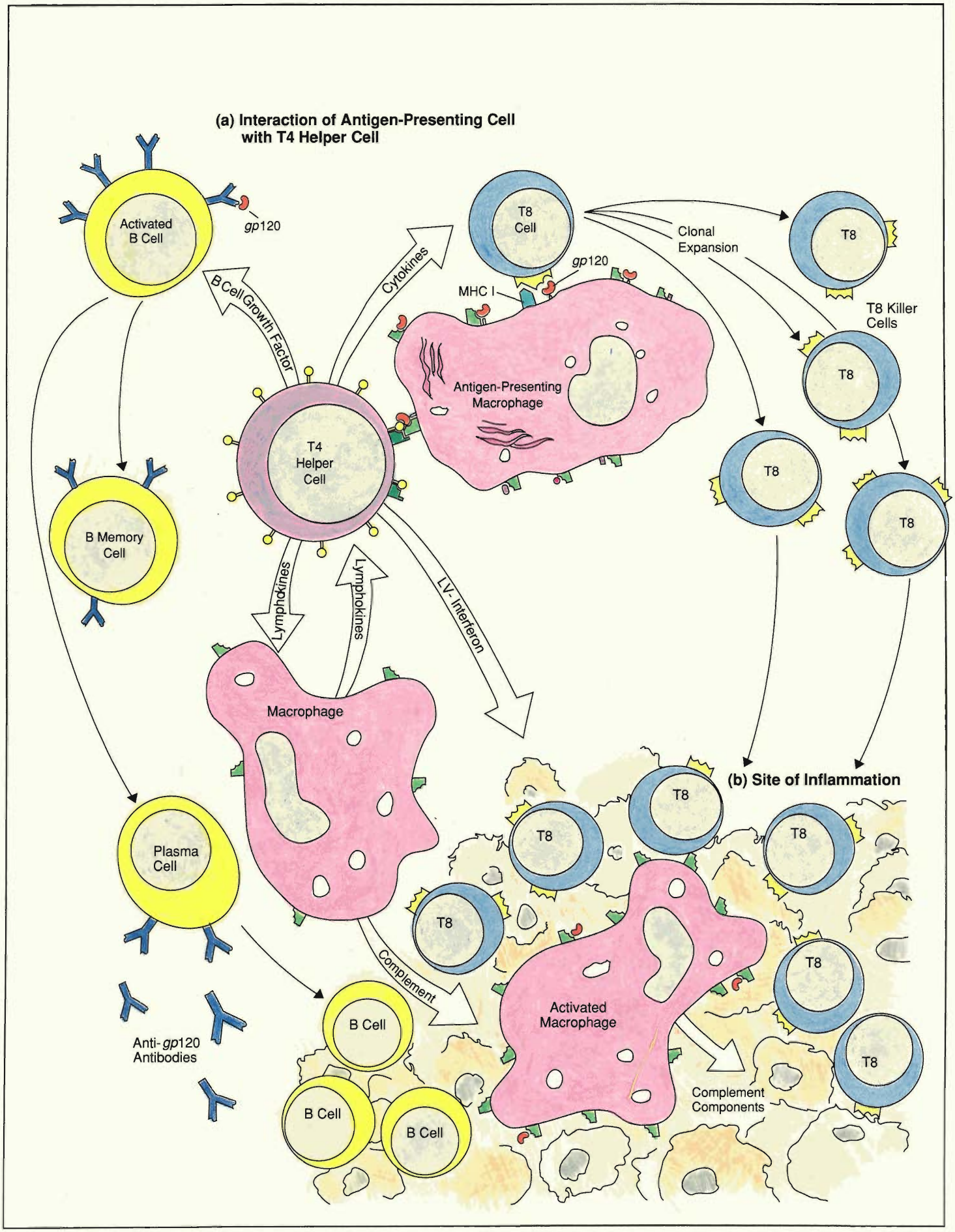
ANTIGEN PRESENTATION TO T4 CELLS

Fig. 4. (a) A macrophage engulfs a foreign monocyte infected with HIV. Some virus particles have budded into a vacuole in the infected monocyte. (b) The engulfed cell is enclosed within a vacuole of the macrophage where it is partially digested by lysosomes and other enzymes. For purposes of illustration, some intact foreign antigens are shown inside the vacuole but, in reality, antigen is broken down into much smaller pieces. Foreign antigens as small as eight amino acids in length, when presented on the macrophage surface, may initiate an immune response. The large vacuole breaks up into smaller and smaller vacuoles that bring the foreign products to the cell surface where they are either released or are presented on the surface in conjunction with MHC II antigens produced by the macrophage. (c) Finally, the macrophage is shown presenting the foreign antigens, in this case *gp*120, to a T4 helper cell. The blowup shows the dual recognition by the T4 cell's receptor CD4 of both MHC II and *gp*120. Note that CD4 appears in conjunction with TCR (T cell receptor) and both are involved in the recognition of MHC II antigens on macrophages and other cells. The dual recognition by the T4 cell of both the self antigen MHC II and the foreign antigen stimulates the T4 cell to orchestrate a defense against the foreign invader.



(b) Macrophage Digests Infected Foreign Cell and Presents Foreign Antigens on the Surface


T4 Cell
CD4
gp120
TCR
MHC II
Antigen-
Presenting
Macrophage
gp41
gp120
p17/18
p9, p7
p24/25
RNA
T4 Helper Cell
(c) Macrophage Presents
Antigen to T4 Helper Cell
Golgi
Vacuole with
Foreign Antigens
Lysosome


(a) Interaction of Antigen-Presenting Cell with T4 Helper Cell
Activated B Cell
gp120
B Cell Growth Factor
Cytokines
T8 Cell
gp120
MHC I
Clonal Expansion
T8
T8 Killer Cells
T8
Antigen-Presenting Macrophage
T4 Helper Cell
T8
T8
B Memory Cell
Lymphokines
Lymphokines
LV-Interferon
Macrophage
(b) Site of Inflammation
T8
T8
T8
T8
T8
T8
Plasma Cell
Complement
Activated Macrophage
T8
Anti-gp120 Antibodies
B Cell
B Cell
B Cell
Complement Components

INTERACTION OF MACROPHAGES AND LYMPHOCYTES

Fig. 5. (a) Immune responses induced by antigen-presenting cells. As in Fig. 4, an antigen-presenting macrophage displaying MHC II and foreign antigen interacts with a T4 cell carrying antigen-specific receptors. In this case the foreign antigen is *gp*120. The interaction produces a series of immune responses with the T4 helper cell as the central player, sending out chemical messages to B lymphocytes, T8 killer lymphocytes, and macrophages. At the upper left, a B cell is activated by the binding of *gp*120 to an anti-*gp*120 antibody on its surface. B cell growth factor from the antigen-driven T4 lymphocyte nonspecifically stimulates the activated B cell to proliferate and mature into memory clones and antibody-secreting plasma cells. A T8 killer cell (top center) is carrying an antigen-specific receptor that recognizes MHC I and *gp*120 on the antigen-presenting macrophage. The T8 cell also receives chemical messages (cytokines) from the T4 helper cell. Both signals work in conjunction to stimulate proliferation and maturation of the T8 cell either into T8 killer cells that travel through the body and destroy infected cells with cytotoxins or into T8 memory cells. In addition, the T4 cell secretes lymphokines that enhance the ability of macrophages to engulf and destroy infected cells and that stimulate the macrophages to produce some of the so-called complement proteins, which stick to and help destroy foreign invaders nonspecifically. (See Fig. 11 for some functions of complement and antibodies.) Lentiviral infections cause chronic stimulation of immune responses shown here. They, in turn, lead to proliferation of lymphocytes and chronic inflammation of lymph nodes, joints, and other organs containing infected macrophages. (b) The invasion by immune cells at a site of inflammation. One specific response associated with the lentiviral infection of sheep, called visna-maedi, is the secretion by T4 lymphocytes (see figure) of a unique γ-like interferon (a cytokine released by virally infected cells or lymphocytes to protect other cells from viral infection). The γ-like interferon seems to suppress viral replication and at the same time induces a persistently high expression of MHC II and some viral antigens on the surface of infected macrophages. The persistent expression of both MHC II and viral antigen is involved in the overactivation or dysregulation of the immune system and inflammation of lymph nodes and infected organs characteristic of lentiviral infections. Similar mechanisms of inflammation for HIV have not been thoroughly investigated.

a multi-leveled, self-controlled defense against the viral invaders.

Monocytes and macrophages are primary targets of all lentiviral infections especially those of nonprimate species. As we will see below, once infected by a lentivirus, the macrophages chronically stimulate the immune reactions shown in Fig. 5, which, in turn, lead to the abnormal accumulation of immune cells and chronic inflammation characteristic of lentiviral diseases.

In human and nonhuman primates the clinically relevant target of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) is both the monocytes and the cell that we singled out as a key player in the immune system, the T4 helper lymphocyte. A progressive decline in the number of T4 cells is correlated with the progression of AIDS from infection to death. We will address the mystery of that decline later. Here we focus on the mechanism by which the virus enters the cell because it involves the surface receptors we have already met in Fig. 4.

It has been found that HIV and SIV infect T4 cells by binding to a particular protein receptor present in great abundance on the surface of those cells, namely *CD4*. As shown in Fig. 4, the normal function of CD4 is to bind to MHC II on antigen-presenting macrophages and thereby stabilize the interaction between T4 cells and macrophages. CD4 is thus part of the MHC recognition network that assures the body of a controlled destruction of its own cells when they are either infected or tumor-prone. Unfortunately, when HIV meets a T4 helper cell, its envelope glycoprotein *gp*120 binds to CD4. The stem (transmembrane protein *gp*41) attached to *gp*120 then inserts itself into the cell membrane, the viral and cell membranes fuse, and the virus dumps its genetic contents into the interior of the cell (Fig. 1). In other words, the virus takes advantage of the recognition network in the immune system to gain entry into cells that are attempting to fight off foreign invaders.

Further research now makes it appear that CD4 receptors are also present on various circulating monocytes, fixed and wandering macrophages, bone-marrow stem cells, B cells, and some T8 killer cells (Fig. 3a). Thus, although it was first thought that HIV is a disease of only the T4 cells, it now appears that their central role in AIDS is mediated through the infection of monocytes and macrophages as well as their bone marrow stem cells.

In addition, studies on the basic science of the immune system have recently provided evidence for an interconnective system between it and the central nervous system. Lentiviral infection of sheep, goat, and nonhuman and human primates have demonstrated strains of the virus that are more neurotropic, that is, more capable of producing neurological disease. Furthermore, studies in the emerging field of neuroimmunology suggest that the two systems have a highly interactive nature. Cell receptors and interactive chemical messages (cytokines) common to both systems are continually being discovered. Numerous reports continue to suggest that HIV, like the other animal lentiviruses, affects the nervous system. This may occur through direct infection of resident brain macrophages (microglial cells), which subsequently alter neural function, or possibly through di-

rect infection of neural cells via CD4 or other receptors common to both the immune and nervous systems.

Very little is currently known about the extent and effect of lentiviral infection in the various macrophage subtypes in the body's reticuloendothelial system. Infection of some or all of them may be related to different clinical manifestations of lentiviral disease seen in various species. In any case, AIDS has many features in common with other animal lentiviral diseases. For that reason, a closer, more comparative look at the spectrum of lentiviral diseases is instructive. But before we do that, let's take a closer look at the process of how the lentiviral infection takes place in the whole animal.

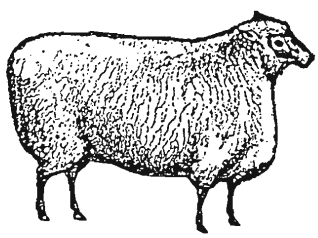
The process of infection. Lentiviral infections are usually introduced into the body by a virally infected foreign cell (Fig. 4). Soon after entry into the host, the infected foreign cell will most likely encounter a strategically located, antigen-trapping macrophage of the reticuloendothelial system (Fig. 3b). On the other hand, the infection can be introduced as a cell-free virus, which will most likely interact with the lymphatic system (Fig. 3c). Thus an infected foreign cell will generally be removed by a tissue-fixed macrophage, whereas a cell-free virus may infect a lymphocyte. The monocytes or macrophages, although doing their job admirably, are probably infected during their attempts to eliminate the foreign cell or virus.

Once infected, the host's immune cell can begin making or spreading virus. In some tissue-fixed macrophages of various lentiviral infected species, viral replication rates seem to be controlled by a combination of cellular and viral regulatory processes, and the infected cells are usually not destroyed by the infection. However, macrophages infected with certain HIV strains will fuse with neighboring cells, either directly transferring the viral genome or releasing extracellular virus. In some animal lentiviral infections, only infrequently will the macrophages degenerate. In contrast, the infection of other animal species will lead to rapid viral replication and lysis of the macrophages or infected T4 helper lymphocytes. Lysis, or break up, of the cell allows massive release of new virus.

Normal immune interactions shown in Fig. 5 promote the spread of the virus, primarily through cell-to-cell interactions, until a critical majority, if not all, of the susceptible wandering and tissue-fixed macrophages of the reticuloendothelial system are infected. The viral spread may be accomplished through cell fusion, which is facilitated by the binding of *gp*120 to CD4 receptors on neighboring cells. Normal interaction between macrophages and lymphocytes may also allow for cell-to-cell transmission of virus. As newborn viruses bud from the cell surface, they coat themselves with the cell's membrane into which they insert their highly sugar-coated, and thereby disguised, envelope receptors. Consequently, the free viruses are poorly recognized as foreign by the immune system, causing the immune responses against them to be ineffectual. Eventually, the immune system is paralyzed as critical immune cells become victimized or eliminated by the virus.

The Animal Models—Examples of Host-Virus Interactions

For any microbe to be a successful pathogen, it must optimize both its rate of producing disease and mortality in the host and its rate of self-replication and transmission. If the two rates are not in balance, then its extinction will be self-assured through natural selection. The slow viruses produce disease in the host only after years of infection. They occupy unique cellular niches, and they are not readily transmitted from host to host without a direct exchange of infected cells or body fluids that contain free virus. Thus lentiviruses have evolved unusual strategies for transmission to assure their procreative investments. Over the past sixty years a few dedicated veterinary and medical researchers interested in persistent viral diseases of various animal species have built a database from which we can derive a multidimensional account of lentiviruses and their various strategies for survival. The variations on the host-virus survival theme seen in animal models lead us to a deeper understanding of the patterns of replication, disease, and adaptation that eventually create successful host-virus relationships in nature. (Not all animal models will be discussed in this article because two have only recently been discovered and others have not been thoroughly researched.)



Visna-maedi

A lentivirus of sheep, visna-maedi, derives its two-word name from the two distinct sets of clinical symptoms it causes: wasting and shortness of breath. These symptoms are associated with dysfunctions of the central nervous and pulmonary systems. We are starting this discussion of animal models with visna-maedi because it caused an epidemic in a manner strikingly similar to that which produced the current worldwide AIDS epidemic. Much like human AIDS, visna-maedi reached epidemic proportions in Iceland in the 1940s, about a decade after the virus was inadvertently introduced into that coun-

BUDDING OF VISNA-MAEDI AND HIV

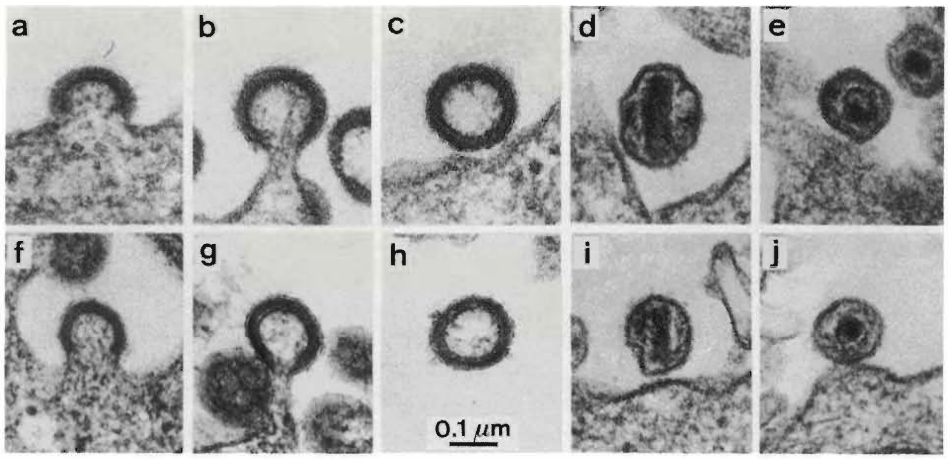


Fig. 6. Transmission electron micrograph of thin sections of cells infected with HIV (top) and visna-maedi (bottom). (a, b, f, and g) Virus particles bud at the plasma membrane. (c and h) Immature, extracellular virus particles have not yet assembled viral cores. (d and i) Mature, extracellular virus particles have bar- or cone-shaped cores. (e and j) From other views, the cores of mature, extracellular virus particles have condensed, circular, eccentric shapes. (Photograph courtesy of Dr. M.A. Gonda, Program Resources, Inc., NCI-Frederick Cancer Research Facility. Reprinted from Gonda et al., *Science*, 227:173-177.) Copyright 1985 by the AAAS.

try. In 1933 the Icelandic government imported twenty karakul sheep from a farm near Halle, Germany, with the intention of creating new types of wool through crossbreeding with native Icelandic herds. Small outbreaks of the visna-maedi disease occurred in the late 1930s, and by 1952 over 150,000 sheep had died. In 1957 an Icelandic physician, Bjorn Sigurdsson, reported that a filterable agent (a feature that distinguishes viruses from other pathogens) was responsible for the disease. He introduced the term "slow virus infection" to distinguish this viral disease from other more acute ones.

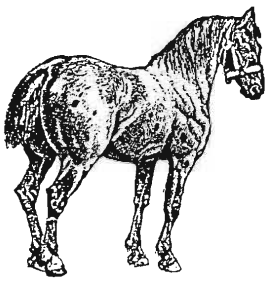
The disease, unlike human AIDS, is usually transmitted among adult sheep through respiratory secretions that contain infected macrophages. (HIV-infected macrophages have been obtained from the lungs of human AIDS patients, although they do not seem to be a major mechanism of transmission.) The vast majority of lambs born to infected ewes are uninfected at birth but become infected after initial suckling of colostrum from the infected mammary glands of the ewe. The visna-maedi virus replicates at the site of entry, generally the lymphoid tissues of the nasal, oral, and upper-respiratory tract, and subsequently spreads via the lymphatic system, the bloodstream, or the cerebrospinal fluid (the fluid in the nervous system). The virus-infected monocytes and macrophages localize in various target organs and cause inflammation and specific pathologies in the lungs, brain, joints, mammary glands, and blood vessels. When first introduced into a nonadapted host (a host that manifests the disease induced by the pathogen as opposed to a host that merely acts as a carrier), visna-maedi leads to high rates of morbidity and mortality. After three or four years, most infected nonadapted sheep reach highly diseased states or die. Soon after infection lambs show apparent ill thrift, that is, poor weight gain and abnormalities in muscle development, skin, hair, and central nervous system functioning. In addition some lambs and adult sheep are subject to opportunistic viral and bacterial infections that add to the systemic clinical signs.

The opportunistic infections associated with visna-maedi, however, do not appear to be as life threatening as in human AIDS. Infections with visna-maedi virus can also lead to subclinical, persistent virus-carrier states, as exemplified in the apparently healthy imported karakul sheep. The persistent viral-carrier state is an example of a successful evolutionary adaptation for lentiviruses with their hosts. There is evidence that certain breeds of sheep are more likely to develop primarily carrier states, whereas others seem to have a genetic predisposition for expression of the visna-maedi disease.

The pathogenesis of visna-maedi and AIDS share a number of similarities. Figure 6 shows the similar budding of both viruses from infected cells. In both diseases proviral DNA is carried covertly in monocytes and macrophages; very few cells circulating in the bloodstream are infected (for HIV, possibly one in ten thousand); and the immune reactions of Fig. 5 are chronically induced by infected, antigen-presenting macrophages. The chronic reactions lead to proliferation of lymphocytes and inflammation in the lungs, the central nervous system, and joints (Fig. 7).

There are also significant differences. The profoundly deficient immune state characteristic of AIDS is not observed

in visna-maedi, presumably because visna-maedi virus is confined to monocytes and macrophages and does not noticeably infect or deplete T lymphocytes. Also, the blood from visna-maedi infected animals is not particularly infectious. In contrast, the human AIDS virus does replicate in T lymphocytes circulating in the bloodstream to the extent that cell-free virus and viral antigens are easily detectable in the blood. Consequently the blood and other body fluids of people with AIDS are infectious. In particular, the presence of HIV particles in the blood is detected in HIV-infected individuals both soon after infection and during the later stages of AIDS. This characteristic, as well as the structure of the human placenta, make the human AIDS virus more likely to infect the fetus. Another closely related species, goats, also have a similar lentiviral disease called caprine arthritis encephalitis. The disease affects primarily the joints, central nervous system, and occasionally the lung. Both the virus and its pathogenesis are very similar to visna-maedi.

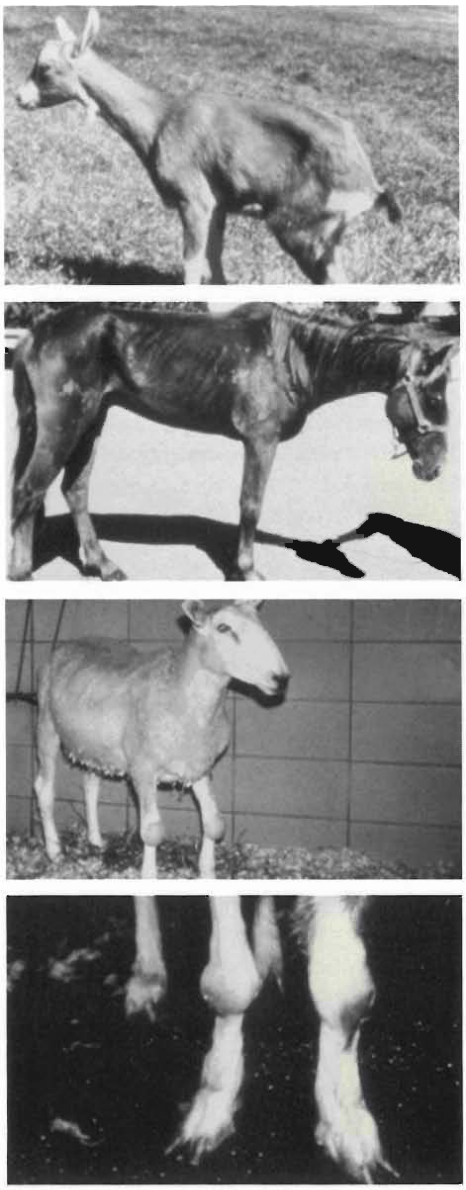


The EIA virus

The earliest known AIDS-like virus is the one that causes equine infectious anemia (EIA), known colloquially as swamp fever. This lentiviral disease occurs in horses and other members of the Equidae family and has been diagnosed worldwide.

The EIA virus is transmitted primarily through blood carried by the biting mouth parts of horse flies and deer flies. This is a particularly efficient transmission process for the virus due to the gregarious social behavior exhibited by the animal hosts. The flies act like flying hypodermic needles, serving solely as mechanical transmission vectors (that is, they physically carry the virus but the virus never replicates in these vectors). The knife-like, slashing mouth parts of the flies cause bites that are painful and elicit host responses, such as tail flicks and shudders, which interrupt the flies's feeding behavior and cause them to move from one horse to another. In this way the virus is disseminated within a herd. This efficient transmission mechanism has evolved for the selection of EIA viral strains that replicate rapidly to high levels of cell-free virus in the blood and are therefore readily transported and transmitted by flies from host to host. Unlike visna-maedi, which results in little cell-free virus in infected sheep, as many as ten million virus particles per milliliter of blood can be found in recently infected horses. The EIA virus, also in contrast to visna-maedi, has been demonstrated to cross the placental barrier and cause fetal infection, although both sheep and horses have placentas containing six functional barriers between the mother and the fetus. The outcome of fetal infection, which is variable and dependent both on the gestational period and stage of infection, leads to either spontaneous abortion or an infected newborn.

The response of adult horses to infection varies, depending on an as yet poorly described host-resistance factor (or factors), viral virulence, and environmental factors, such as stress associated with weather, shipment, and breeding. Infected horses go through various stages of disease. The acute stage, most often associated with the initial exposure to the virus, includes fever and hemorrhages throughout the body within seven to thirty days after exposure. Acute EIA is probably caused



CLINICAL MANIFESTATIONS OF LENTIVIRAL DISEASE

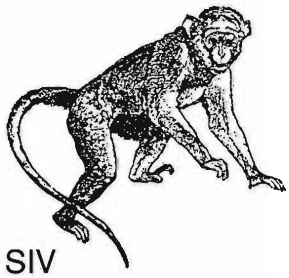
Fig. 7. Animal photographs showing typical manifestations of lentiviral diseases. Wasting is seen in a goat with caprine arthritus encephalitus (a very close cousin of visna-maedi), and a horse with EIA (top two). Swollen knee joints are seen in a sheep with visna-maedi and another goat with caprine arthritis encephalitis (bottom two). (Photographs by Opendra Narayan of John Hopkins University.)

by the initial high rate of viral replication in monocytes and macrophages, which leads to the destruction of these cells. Large amounts of free virus are present in the blood, but antibodies are not produced rapidly enough to inhibit the spread of the infection, presumably because the rapid destruction of macrophages leaves insufficient time for these antigen-presenting cells to signal the lymphocytes. Subsequently, antibodies that neutralize the virus are made. At the same time, however, other virus variants, the so-called escape mutants that are not neutralized by the antibody, are also produced through viral replication. Details of this process will be presented later.

The less acute stages of EIA (Fig. 7) are the more commonly diagnosed forms of the disease. Clinical symptoms include loss of weight, loss of appetite, anemia, exercise intolerance, weakness, and fluid accumulation (edema). These symptoms recur in biweekly cycles for one to four months. The frequency and severity of the clinical episodes usually decline in time, so that approximately 90 per cent of all that do occur have occurred within one year of infection. The earliest deaths due to EIA usually do not occur until four weeks after infection, at which time antibodies to the EIA virus are present in the blood of the animals. Chronically ill horses may suffer acute episodes at unpredictable times. Studies have shown that these recrudescent episodes are sometimes induced by the administration of immune-suppressing drugs or other environmental factors that lead to changes in the immune system. More recently, experimental inoculation with the EIA virus of young Arabian horses suffering from a combined immunodeficiency syndrome characterized by the absence of T and B lymphocytes caused a rapid, virus-induced anemia and death. Both studies demonstrate that horses must have some aspects of a well-functioning immune system to keep the virus under control. Further studies into these controlling mechanisms should provide much insight into successful host-viral interactions.

Many clinical signs of EIA are attributable to changes induced by immune reactions to viral antigens. For example, the anemia (loss of red blood cells) generally associated with morbidity and mortality is caused by the presence of a hemagglutinin-like protein on the surface of free virus that has an affinity for red blood cells. Once a virus binds to these cells, antibodies directed against the virus also bind to the red blood cells. This binding stimulates a cascade of chemical reactions in the blood serum that produce *complement*. These special proteins accumulate around the activated site on the cell and tear or lyse the cell membrane, thereby destroying the cell. Even in the absence of antibodies, viruses bound to red blood cells will attract and bind complement, resulting in the same destruction. As in visna-maedi disease and AIDS, the immune system is chronically activated and immune cells proliferate or accumulate in all organs of horses dying of the disease. The animals also have hemorrhages and enlarged spleens, livers, and lymph nodes. Most infected horses, however, control their viral infection and exist for many years as healthy carrier animals, serving as a long-term source of the EIA virus, as do the adapted sheep for visna-maedi.

Whether EIA virus infects lymphocytes, as does HIV, is not completely resolved. Cell-free virus in the blood, characteristic of the first stages of EIA, also can occur in humans immediately after infection with HIV and is frequently seen in the later stages of AIDS. Since human placentas have only three functional barriers between the fetus and the mother, compared to the six in horses, HIV has a greater chance of infecting human newborns than does the EIA virus have of infecting a foal.



SIV

The Simian immunodeficiency virus, another prototypic lentivirus, infects numerous nonhuman primate species in central and western Africa. This group of viruses, originally isolated from captive rhesus monkeys in 1985, represents the closest known relatives of the human AIDS viruses. (In particular, SIV is more closely related to HIV II than to HIV I.) SIV and the HIVs have very similar genomes and similar biological and antigenic properties (for example, when the purified viral proteins of each virus are injected into laboratory animals, they induce the production of antibodies that recognize each other's proteins).

The natural history of SIV in healthy, free-roaming African primates is best exemplified by the strains found in the African green monkey, the sooty mangabey, the mandrill, the deBrazza monkey, the Sykes monkey, the talpoins, the quereza colobus, and baboons. All of these strains of SIV live in their respective naturally adapted African primates without producing overt clinical disease. Their presence in the naturally adapted species is detected by the isolation of viral particles from cultured blood lymphocytes and by the presence of antibodies circulating in the bloodstream. Detailed studies of the natural transmission in the adapted species are lacking. However, serological tests of the animals in their native habitats show that not all members of a species have antibodies. Further studies are underway to test susceptibility to

the virus of African green monkeys that do not test positive for the antibody. It would be presumed that through various exchanges of tissue, blood, and bodily fluids during species-specific behaviors, such as territorial and reproductive encounters, as well as integration of the virus into the host genome, the virus is assured continued survival within the species. The evidence of numerous genetically distinct SIVs infecting numerous nonhuman primate species strongly suggests that the primate lentiviruses have existed for long evolutionary times.

In the late 1970s and early 1980s, a number of primate centers began experimental manipulation of African primates to study the biology of lymphoma and leukemia as well as leprosy. Unbeknown to those researchers, the studies provided the arena for the unnatural transmission of various host-adapted African SIVs to nonadapted Asian primates, such as rhesus and pigtail monkeys. Some months to years following the studies, a wasting, debilitating disease, characterized by opportunistic infections as well as tumors, occurred among the primate colonies involved. That somewhat unfortunate event had the serendipitous outcome of creating a very useful primate model for studying AIDS.

If the scenario just described for nonhuman primates is extended to humans, it would suggest that the AIDS epidemic had its origins in Africa, where either ancestral co-evolution or natural virus transmission from adapted nonhuman primates to humans created populations that were at various stages of natural adaptation with an ancestral AIDS-like virus. Whatever the evolutionary route, the result was a worldwide population of susceptible and nonsusceptible humans capable of transmitting the AIDS virus to other humans elsewhere. The cosmopolitan nature and urbanization of Africa and various third-world nations in the 1970s and 1980s then facilitated the transmission of the virus from adapted human HIV carriers to nonadapted members of the species, just as the well-intentioned importation of sheep to Iceland allowed the transmission of visna-maedi.

One or all of the following viral-anthropological schemes may have occurred in Africa and led to the current worldwide epidemic. An ironic possibility is that the AIDS virus was additionally introduced to less adapted Africans through the intense interaction between native Africans and their indigenous primates initiated by the scientific demand in the United States and elsewhere for wild, caught primates to be used in human hepatitis studies in the late 1950s and the 1960s. Anyone experienced and working with primates can tell you of the numerous combative instances that occur during capture. The deep cutaneous wounds from bites and scratches that often occur would serve nicely for introduction of cell-free virus or virally infected cells into the human population. (It is interesting to note that all isolated SIVs grow readily in vitro and kill cultured human T4 helper cells. Therefore, identical biosafety precautions are taken when handling SIV as when handling HIV.)

Another possible transmission route from nonhuman primates to humans involves more customary cultural practices—such as ancestral tribal rituals or hunting primates as a source of sustenance.

An equally plausible explanation for the presence of HIV in central Africa would be the parallel evolution of an HIV-like, host-adapted SIV and modern man. Central Africa is considered by anthropologists to be the evolutionary birthplace of man. Perhaps some ancestors of modern man such as the chimpanzees or gorillas were co-evolving with a host-adapted, HIV-like virus, while others were evolving with a host-adapted, HIV-like SIV, and still other ancestral human primates were not evolutionarily linked to either SIV or HIV. Thus, the stage would be set for very susceptible, moderately susceptible, and less susceptible populations of humans as one sees in the primates and the other animal species previously described. Evidence for such distinct populations will await more extensive and detailed studies.

Ongoing studies in primate centers suggest that, like HIV, SIV can be transmitted through blood containing infected cells or free virus. Whether transmission occurs primarily through semen or other body fluids has not yet been completely characterized. When cell-free SIV is introduced into the vagina of a normal rhesus monkey, the monkey becomes infected as efficiently as when these animals receive virus in the blood. Following entry into the host, the human or simian AIDS viruses infect T4 lymphocytes, monocytes, and macrophages of the monkey, again through the CD4 receptors on the surfaces of these cells. In contrast, recall that the target cells of visna-maedi and the EIA virus are primarily macrophages.

The clinical signs of nonadapted SIV-infected primates parallel those of HIV-infected humans more closely than the other animal models previously described. A prominent swelling of the lymph nodes throughout the body, diarrhea, fever, lack of appetite, depression, inactivity, loss of weight, and neurologic complications characterize the early and later stages of the disease. As the disease progresses, the clinical manifestations caused by the initial viral infection become difficult to identify because they become mixed with those caused by systemic, opportunistic infections that are often fatal.

Also like HIV infection in humans, SIV infection of nonadapted primates leads to progressive loss of T4 helper cells, resulting in severe immune sup-

Table 2

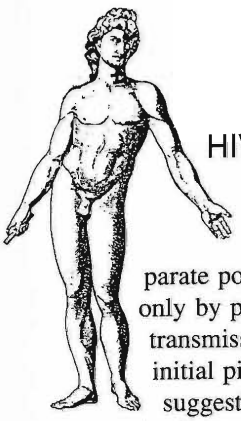
Clinical Manifestations of Lentivirus Infections in Natural Hosts

Host	Lentivirus	Disease Description
Sheep	Visna maedi, progressive pneumonia virus	Generalized wasting Chronic encephalomyelitis Progressive lethal pneumonia Spasticity Paralysis Lymphadenophathy (swollen lymph nodes) Opportunistic infections
Goat	Caprine arthritis encephalitis virus	Generalized wasting Chronic leukoencephalomyelitis Progressive arthritis Osteoporosis Paralysis
Horses	Equine infectious anemia virus	Fever Intermittent anemia General proliferation of lymphoid cells in retriculoendothelial system Glomerulonephritis
Cow	Bovine immunodeficiency-like virus	Persistent lymphocytosis Lymphadenophathy Wasting Central nervous system lesion
Cat	Feline T-lymphotrophic virus	Immunodeficiency-like syndrome Generalized lymphadenopathy Leukopenia Fever Anemia Emaciation
Monkey	Simian immunodeficiency virus (SIV)	Immunodeficiency Neutropathologic changes Wasting Opportunistic infections
Human	Human immunodeficiency virus (HIV)	Immunodeficiency Opportunistic infections Lymphadenopathy Encephalopathy Kaposi's sarcoma

pression. It is interesting to note that a particular SIV strain taken from adapted primate species rapidly induced (within seven to fourteen days) viral-associated death in unrelated nonadapted species, whereas other SIVs induced death and disease in nonadapted species only after months and sometimes years. Perhaps some evolutionary relationship exists between a species and its overall susceptibility to lentiviral disease.

At this point we can summarize this

discussion by listing the biological hallmarks shared by all the lentiviruses: their host range tends to be genus-specific rather than species-specific; their transmission occurs horizontally through blood, milk, other body fluids, and inflammatory exudates containing either infected lymphocytes, infected macrophages, or free virus; they cause lifelong infections in monocytes, macrophages, and lymphocytes; they replicate irregularly or continuously at enhanced or restricted rates; and they may or may not cause disease after variable or often prolonged periods of subclinical infection, depending on various virus and host factors. Table 2 lists the clinical manifestations of the various lentiviral diseases.



The appearance of AIDS in disparate populations connected only by probable routes of transmission was among the initial pieces of evidence suggesting an infectious cause to the forthcoming worldwide epidemic. First described among homosexual men in June 1981, AIDS was recognized among intravenous drug users and Haitians the following year and among recipients of blood or blood products, infants born to mothers at risk, heterosexual sexual partners of patients with AIDS, and Africans by early 1983. Thus the search for a blood-borne infectious agent resulted in the discovery of the human immunodeficiency virus in 1983. Thus far two strains have been identified, HIV I, which appears to be the predominant virus affecting Africa and the western world, and HIV II, more closely related to SIVs and currently responsible for infecting various West African populations, albeit at a slightly attenuated mortality rate.

Humans infected with HIV generally develop an early flu-like syndrome that includes fever, malaise, loss of appetite, sore throat, night sweats, generalized swollen lymph nodes, and diarrhea. As the disease progresses over the next one to seven years, the lymph nodes remain enlarged and the circulating T4 cell population in the body progressively declines. The decline leaves the body vulnerable to a large number of opportunistic infections, such as a rare type of protozoal pneumonia, a nervous system infection due to a parasite of cats, and an unusual type of cancer of blood vessel origin (Kaposi's sarcoma). The infected human generally succumbs to one of these opportunistic infections.

The virus is spread predominantly in blood and blood products and has been discovered in saliva, breast milk, and cerebrospinal fluid. Soon after the initial infection and at various unpredictable times during incubation and throughout the disease, large amounts of cell-free virus are found in blood. At these times the immune system appears to be inactive as the virus increases its chances for transmission through mediums such as serum, plasma, or breast milk.

The virus has been found to infect and is recovered from T4 cells, monocytes, and macrophages in blood, lung, and brain tissues. Current studies suggest that various cells of the central nervous system can be infected at a low level. Infection of the lymphocytes generally leads to rapid cell death and the release of large numbers of cell-free virus particles. Infection of monocytes and macrophages, on the other hand, leads to an event in which variable numbers of virus particles are found budding within the cell without killing it.

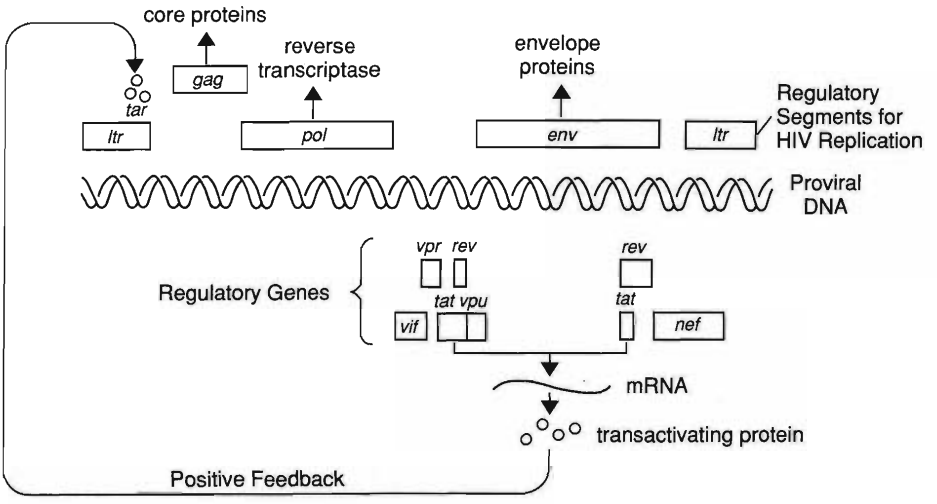
All human races appear susceptible to infection and the subsequent development of fatal immunosuppression. The previously mentioned carrier state of other animal lentiviruses is not as common in HIV. Although less pathogenic viruses have been isolated, extensive investigations into adapted native African populations have, to date, not been reported. We know that some individuals have been infected for greater than nine years with no identifiable symptoms, but we have no substantial database or explanation as to how this occurs. Investigations currently underway to study the genetic predisposition to the virus in the human population should eventually yield general statements about variations in susceptibility to disease. Our discussion suggests that virus-adapted African subpopulations should already exist in some parts of Africa.

Mechanisms of Viral Persistence

Viral persistence, or the inability of the host to completely rid itself of the virus, may be the outcome of a number of viral properties. First, the virus may disguise itself or mimic properties of the host's normal cells. Second, the virus may infect a small subset of cells situated in the brain, reproductive organs, and parts of the eye and joints that are immunologically privileged, that is free from the usual scrutiny of the immune system. Third, the virus may paralyze or destroy certain immune functions directly responsible for its elimination. Fourth, it may integrate itself into the genetic make-up of the cell, thereby insuring itself subsistence as long as the normal cell is not eliminated. Fifth, the virus may have genetic controlling elements that regulate and limit its expression. Lastly, the virus may have an ability to continually change itself on a regular basis such that the immune system is never able to "catch up." Although some of the six strategies just mentioned are found in other

Table 3

Genetic Map of HIV



The HIV genome contains about 10,000 nucleotide pairs. Nine genes are shown here, arranged in sequence along the viral DNA. (Since protein-coding regions can be read in three ways, a number of genes can overlap on one DNA segment.) The genes are flanked by long-terminal-repeat (*ltr*) regions, noncoding regions that initiate expression of viral genes. The *gag*, *pol*, and *env* genes code for core proteins, reverse transcriptase (and other enzymes), and envelope proteins, respectively. HIV also contains an unusually large number of regulatory genes, described below.

Regulatory Genes

tat — A positive regulator that amplifies viral replication. The *tat* gene does this by producing a transactivating protein that stimulates a transacting response sequence (*tar*) in the *ltr* region of the genome. The *tar* sequence is included in every mRNA transcript of every HIV gene. Thus, *tat* boosts production of both regulatory and structural viral proteins, including its own protein, and can amplify viral replication by a factor of a thousand.

rev — A differential regulator that enables selective production of either regulatory proteins or new virion components by a transacting antirepressive mechanism. The *tat* and *rev* genes can counteract each other to produce steady-state levels of *tar* and *rev* regulatory proteins.

nef — A negative regulatory factor that suppresses viral expression.

vif — A viral infectivity factor whose protein product enhances the ability of new virus particles to fuse with and enter uninfected cells.

vpr — This segment has an unknown function, but codes for protein.

vpu — This segment has an unknown function, but codes for protein.

persistent viral infections, such as Herpes viruses (I, II, and cytomegalovirus) adenoviruses, and influenza viruses, only the lentiviruses appear to have adopted them all. In the following sections we will bring together data on the mechanisms of lentiviral persistence from the previously described animal models and from recent studies of HIV-infected humans.

Cellular-Viral Regulation. The slowness or persistence of lentiviral infections reflects the fact that viral replication in T lymphocytes and monocytes is often minimally productive—viral replication generally takes place at a very slow rate. It is, however, the limited production of viral antigens that allows the infected cells to go unnoticed by the immune system for long periods. Alternatively, the virus's life cycle stops at the proviral DNA or the RNA transcription stage. Infected cells that are invisible to the host's immune defenses, yet capable of transmitting the virus from cell to cell, are sometimes referred to as the "Trojan Horse" phenomenon.

As mentioned in our earlier discussion of the immune system, immunological signals that activate T cells and signals that initiate the maturation and differentiation of monocytes into macrophages are the norm in daily immune functions. It is these signals, however, that seem to initiate, enhance, and control lentiviral replication within infected cells. Although viral replication does not necessarily kill infected cells, the presence of the virus seems to impair the cell's functioning and to preclude the cell's ability to eliminate other foreign invaders. Moreover, activation of the latent viral state appears to occur at just those times when viral replication will assure transmission of the virus to new host cells.

The ability of lentiviruses to go from a state of "controlled hibernation" to a state of "controlled activation" following

SYNCYTIUM — A GIANT MULTINUCLEATED CELL

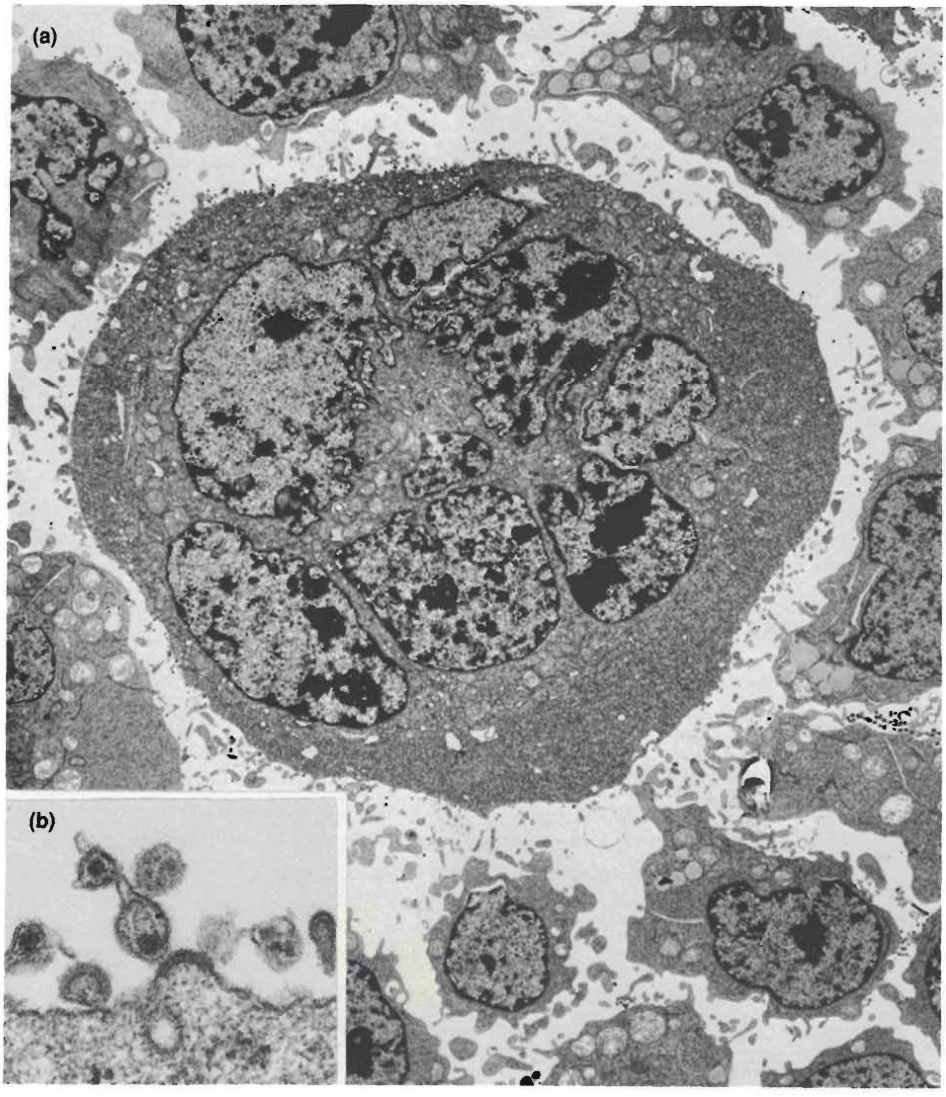
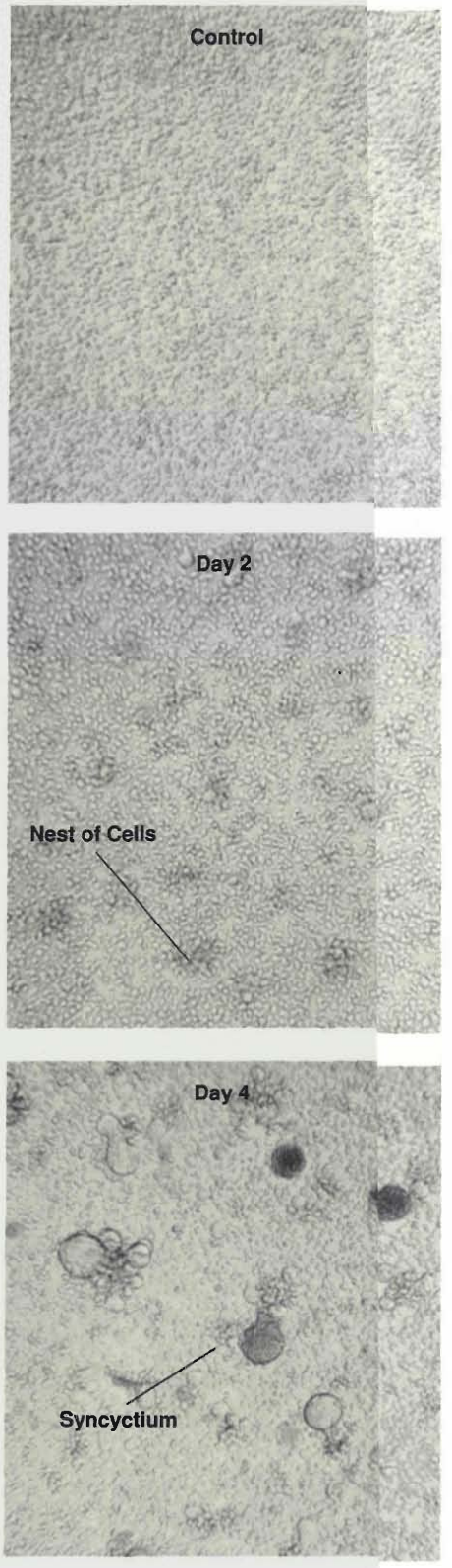


Fig. 8. (a) Transmission electron micrograph of a giant multi-nucleated cell formed in vitro by the fusion of an HIV-infected transformed human T4 lymphocyte with other lymphocytes from the same cell line (magnified 1200 times). The syncytium is rapidly producing virus particles. (b) The budding of viral particles in (a) (magnified 25,000 times). The transformed, or tumor, cell line shown here and developed by Robert Gallo produces large numbers of HIV particles without undergoing cytolysis and has therefore been instrumental in AIDS research. (Photograph by Kunio Nagashima, NCI-Frederick Cancer Research Facility.)

stimulation of the immune system must be related to their unusually large number of regulatory genes. These "extra" genes, which were either evolved independently by the virus or were pirated from the host's immune cells, appear to work in concert with the host cell's machinery and extracellular signals to limit or enhance viral gene expression as needed for survival of the virus. Table 3 lists the known regulatory genes in HIV, for which the detailed functions are only partially known.

The state of controlled viral replication is lost in all species of AIDS viruses when they are placed in tissue culture. Viral replication takes place rapidly in peripheral blood lymphocytes when stimulated artifically to divide. An infected T4 cell transcribes proviral DNA into several thousand copies of viral RNA, which serve as genomes for new virus particles and templates for production of viral proteins. The redirection of cellular machinery for the massive production of viral components leads to a loss of the normal protein synthesis required to maintain cellular integrity. In addition, the RNA genomes and viral proteins assemble into infectious virus particles, which, in some instances, massively bud from the cell surface, thereby destroying the cell. A single infected cell may produce 500 to 1000 of these. Massive viral replication may occur in vivo as evidenced by detectable levels of viral antigens or infectious cell-free virus circulating in the serum of about half of the AIDS patients at various times during the course of the disease.

Detailed knowledge of the cellular factors controlling the virus life cycle in monocytes and macrophages comes from in vitro studies of visna-maedi virus. The visna-maedi life cycle is highly dependent on maturational factors in these cells. Less differentiated monocytes are more difficult to infect and the viral life cycle stops after proviral DNA is transcribed into RNA. As monocytes age, they are more easily infected, and viral replication proceeds all the way to the production of viral proteins. This regulatory program as-

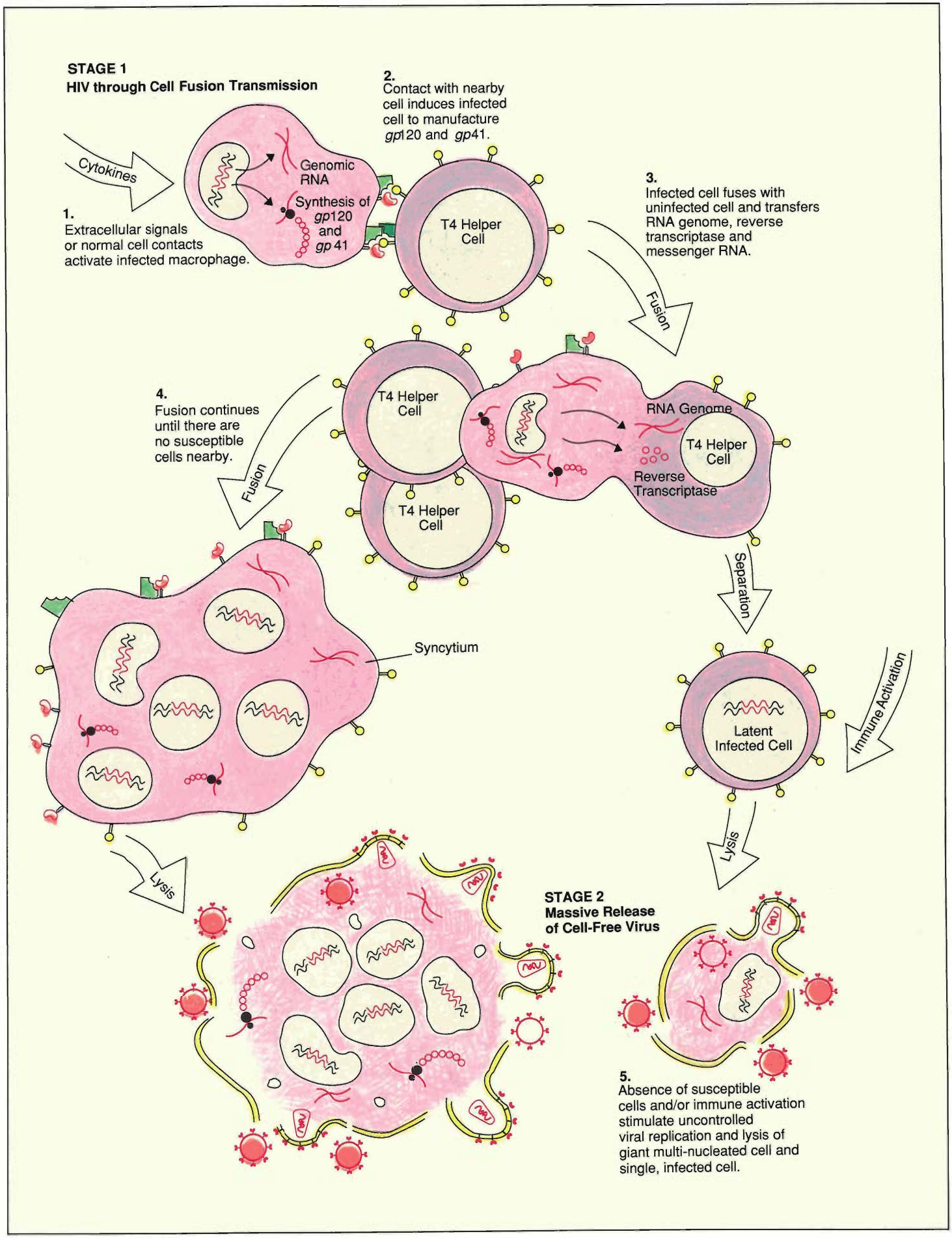


FORMATION OF SYNCYTIA IN MICROASSAY

Fig. 9. Photomicrographs of sequential stages of cell fusion and syncytial formation in the quantitative HIV I-induced infectivity microassay. The top picture depicts normal uninfected cells forming a monolayer. The middle and bottom pictures demonstrate cell-to-cell fusion. Note the cell nests or clusters (arrow) that occur by day two in culture. By day four or five, these cell nests form the typical syncytia described in the text and shown in Fig. 8.

lymphokine, or cell-recognition, receptors, that is, the MHC receptors. These cell-surface receptors receive chemical signals that direct the cells around the body and induce normal immune activity in the lymph system. Normal signals that prepare the various neighboring immune cells to interact with each other seem to activate the HIV envelope genes within the infected cell to produce the "fusigenic" envelope proteins, *gp*120 and *gp*41, that cause cells to stick together and fuse.

What of the second stage? It seems that if the HIV-infected fusigenic cells fail to find neighboring cells susceptible to fusion, a new set of cell-membrane signals induces the viral genes to redirect the cell's machinery toward producing the additional structural components required to assemble new infectious virus particles. The massive production of these new particles, sometimes at the expense of the cell, can be considered a terminal last ditch effort on the part of the virus to infect new cells and thus survive in the host. As virus particles bud from the cell, they strip off pieces of the protective cell membrane. Normally, cell membrane components are constantly being re-formed through protein synthesis to keep up with the everyday import and export of cellular


STAGE 1
HIV through Cell Fusion Transmission
Cytokines
1.
Extracellular signals or normal cell contacts activate infected macrophage.
Genomic RNA
Synthesis of gp120 and gp 41
2.
Contact with nearby cell induces infected cell to manufacture gp120 and gp41.
T4 Helper Cell
3.
Infected cell fuses with uninfected cell and transfers RNA genome, reverse transcriptase and messenger RNA.
Fusion
T4 Helper Cell
RNA Genome
T4 Helper Cell
Reverse Transcriptase
T4 Helper Cell
4.
Fusion continues until there are no susceptible cells nearby.
Fusion
Separation
Syncytium
Latent Infected Cell
Immune Activation
Lysis
Lysis
STAGE 2
Massive Release of Cell-Free Virus
5.
Absence of susceptible cells and/or immune activation stimulate uncontrolled viral replication and lysis of giant multi-nucleated cell and single, infected cell.

TWO-STAGE MODEL OF VIRAL REPLICATION

Fig. 10. HIV regulatory genes, in response to extracellular signals, seem to produce two distinct stages of viral replication that assure survival of the virus in the host. Stage 1. In the presence of CD4-positive cells, the infected cell produces the fusigenic viral proteins, *gp*41 and *gp*120, that cause fusion of the infected cell's membrane with the membrane of a neighboring CD4-positive cell. The viral genome and reverse transcriptase is then transferred to the uninfected cell. The newly infected cell may then separate to become a latent infected cell. Alternatively, it may take part in the fusion process with other nearby CD4-positive cells to form a giant multi-nucleated cell called a syncytium. In this way, the infection spreads slowly with no interference by the immune surveillance system. Stage 2. When no uninfected CD4-positive cells are nearby, the syncytium switches into a state of uncontrolled viral replication, which produces thousands of new infectious virus particles. As these bud from the surface, they tear, or lyse, the membrane and thereby destroy the giant cell. A single latent uninfected cell, when stimulated by extracellular signals, may also undergo uncontrolled viral replication, resulting in lysis of the single cell. The infectious viral particles now encounter immune defenses as they travel through the body to find new infectible cells.

materials. However, the uncontrolled production of 500 to 1000 particles per cell and the holes they create as they bud from localized areas on the cell surface, cause the cell to take on excess extracellular fluids, burst, and die.

The newly created HIV particles, unlike some other viruses, appear to undergo a relatively rapid predetermined decay caused by the spontaneous shedding of the *gp*120 molecule, the molecule that binds to CD4. The shedding is apparently due to the interactive yet weak protein structure of *gp*120. Studies in my laboratory show that the shedding takes from 8 to 15 hours. Hence a race begins to find a new infectible host cell before the virus particle loses its ability to infect. (See "The Kinetics of HIV Infectivity" for a detailed discussion of this process in vitro.) In summary, if the infected cell is locked in a compartment of the body with no direct access to infectible cells and therefore no chance for fusing, the virus programs the cell to produce hundreds of virus particles, which can rapidly diffuse in extracellular fluids or in the bloodstream.

Biological Properties of the Virus.

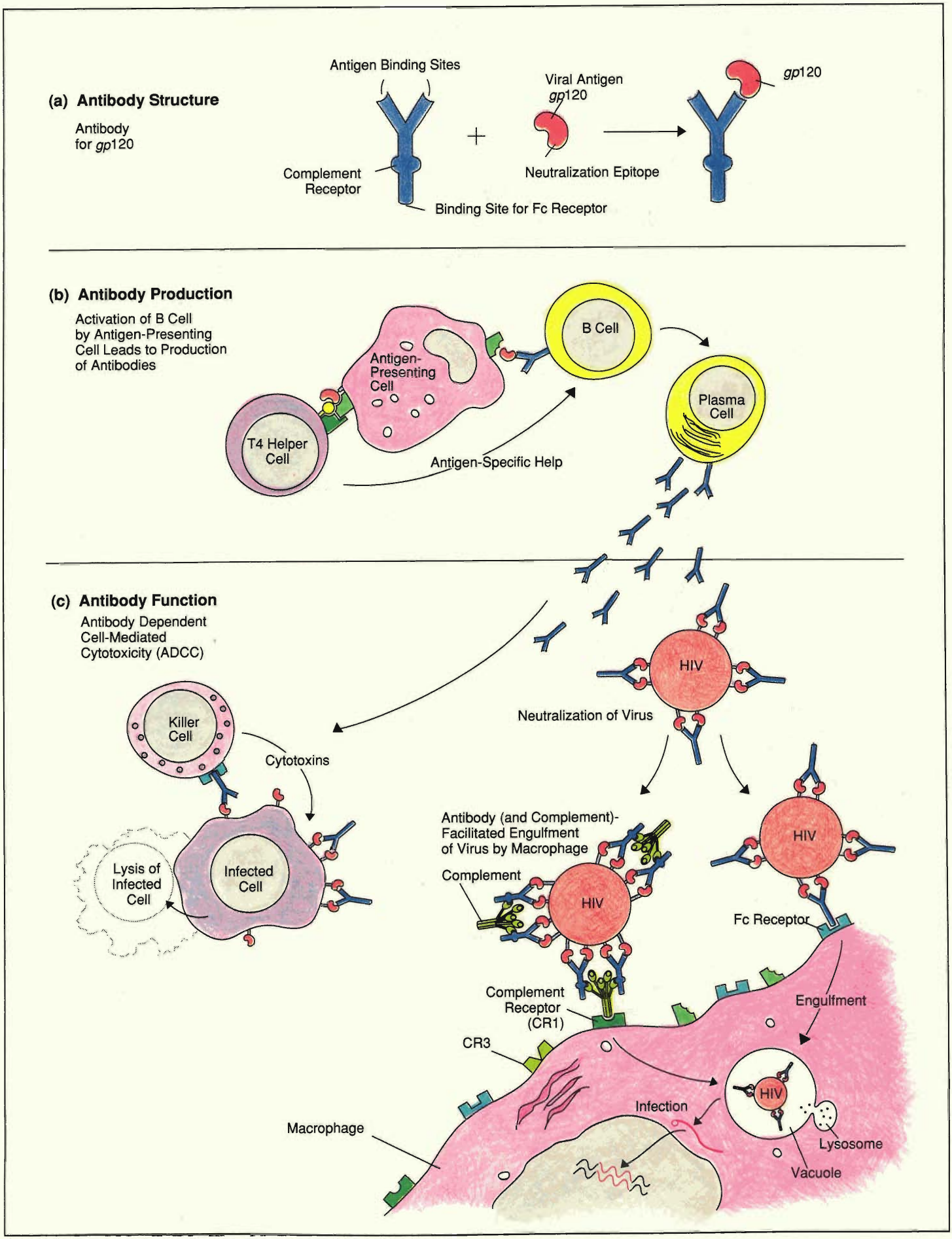
Having discussed *regulatory* processes that help assure presistence of the virus, we now turn to *structural* properties that help the virus escape from host immune defenses. The glycoproteins *gp*120 and *gp*41 forming the envelope of HIV have two biological properties important to the survival of the virus: 1) they contain large amounts of carbohydrate (sugar), which serves to minimize and hide their protein binding sites from the host immune system and 2) they insert themselves next to or within the host cell's own self-recognition proteins, such as the MHC molecules. Both properties help the virus to escape from normal antiviral immune mechanisms previously outlined in Fig. 5.

Antibodies are Y-shaped proteins that neutralize the virus by binding to specific molecular protein shapes, called *epitopes*, on the viral envelope proteins (Fig. 10). In most lentiviruses, almost all neutralization epitopes are highly glycosylated (sugar coated), and these carbohydrate moieties completely conceal neutralization epitopes from immune recognition. As a result, the B lymphocytes are not able to produce highly effective neutralizing antibodies. In the case of HIV, we are a bit more fortunate in that some effective neutralizing antibodies are produced (see "The Search for Protective Host Responses").

In all strains of lentiviruses, some epitopes are variably exposed and induce the production of neutralizing antibodies of very narrow specificity (that is, they recognize only the one viral strain). While the neutralizing antibodies may be effective against the original virus, mutations occur frequently in the genes for the viral envelope and lead to production of new virus particles with rearranged neutralization epitopes. The new particles now escape neutralization by antibodies. This process has been called *antigenic drift*, a term previously coined for influenza viruses, which cause the common cold. The mutational phenomenon is seen in sheep infected with visna-maedi virus, in horses infected with the EIA virus, and in humans infected with HIV.

Moreover, non- or poorly-neutralizing antibodies can *facilitate* the infection of macrophages. The loosely associated virus-antibody complex sticks to an antibody receptor present on the surface of the macrophage. The macrophage then engulfs the virus-antibody complex and thereby becomes infected (Fig. 10). Thus, certain antiviral antibodies produced during the lentiviral infection serve no useful biological purpose and therefore seem to perpetuate rather than eliminate infection in the host.

As previously mentioned, after a host cell has become infected, the viral glycoproteins insert themselves strategically next to or within the MHC antigens normally present in the cell membrane. Since MHC proteins are precisely the surface antigens that cells of the immune system use to recognize each other as self, the viral glycoproteins act very much like a "wolf in sheep's clothing." The net result is a form of molecular mimicry. In particular, recall that the presence of *gp*120 in the membrane of the infected cell allows it to fuse with any neighboring cell that has a


(a) Antibody Structure
Antibody for gp120
Antigen Binding Sites
Viral Antigen gp120
gp120
Complement Receptor
Neutralization Epitope
Binding Site for Fc Receptor
(b) Antibody Production
Activation of B Cell by Antigen-Presenting Cell Leads to Production of Antibodies
B Cell
Antigen-Presenting Cell
Plasma Cell
T4 Helper Cell
Antigen-Specific Help
(c) Antibody Function
Antibody Dependent Cell-Mediated Cytotoxicity (ADCC)
HIV
Neutralization of Virus
Killer Cell
Cytotoxins
Lysis of Infected Cell
Infected Cell
Antibody (and Complement)-Facilitated Engulfment of Virus by Macrophage
Complement
HIV
HIV
Fc Receptor
Complement Receptor (CR1)
Engulfment
CR3
HIV
Infection
Macrophage
Lysosome
Vacuole

ANTIBODY STRUCTURE, PRODUCTION, AND FUNCTION

Fig. 11. (a) Antibodies are Y-shaped protein molecules whose arms contain antigen-specific binding sites. The antibody shown here is specific to *gp*120. The base of all antibodies contains a binding site for the Fc receptor present on macrophages and killer cells. Antibodies also have binding sites for some complement proteins and thereby work in conjunction with complement to coat foreign invaders and attract scavenger cells, which destroy them. (b) Antibodies are produced by B lymphocytes. Here a B cell binds through its antibody receptor to an antigen-presenting macrophage and also receives chemical signals from a T4 helper cell. The combination stimulates the B cell to proliferate and mature into antibody-secreting plasma cells. (c) One function of antibodies is to neutralize viruses; as shown in the figure, they accomplish neutralization by binding to the virus's surface receptors, which prevents the virus from infecting a host cell. The figures also show how antibody-coated virus may bind to the Fc receptors on macrophages, which leads to engulfment and digestion of the antibody-virus complex. If complement binds to the antibody-virus complex, engulfment by macrophages is further facilitated. In the case of HIV, coating of the virus by nonneutralizing antibody may lead to engulfment by and subsequent infection of the macrophage. A second major function of antibodies (bottom left) is to coat infected cells and thereby attract, bind to, and stimulate killer cells, to secrete cytotoxins, which lyse the infected cells. This process is called antibody-dependent, cell-mediated cytotoxicity (ADCC).

CD4 receptor on its surface and then to dump its viral genetic payload into that cell without the virus itself ever being assembled or encountering neutralizing antibodies, killer T8 lymphocytes, or other complex extracellular antiviral moieties that might interfere with the infection process.

Table 4

Factors and Processes Leading to T4 Cell Depletion

1. Accumulation of unintegrated viral DNA.
2. Massive budding of new viruses, leading to breakup, or lysis, of cell membrane.
3. Abundance and maintenance of CD4 receptors on T4 cells, promoting infection, autoinfection, and cytopathic effects.
4. Cell fusion between T4 cells, promoted by complexing of viral envelope proteins with CD4.
5. Infected T4 cells expressing *gp*120 are recognized as *non-self* and destroyed by immune reaction.
6. Binding of free *gp*120 to uninfected T4 cells, leading either to binding of anti-*gp*120 antibodies or to direct attack by cytotoxic cells.
7. Antibodies to *gp*120 cross-react with MHC II expressed on activated T4 cells, and the antibody-coated T4 cells are subsequently destroyed by K (killer) cells.
8. HIV infection of bone marrow stem cells, leading to decreased production of mature T4 cells or HIV infection of a T4-cell subset that is critical to the propagation of the entire T4 cell pool.
9. Secretion by HIV-infected cells of soluble factors that are toxic to T4 cells or secretion of such factors induced by the free virus.

Immune Dysregulation and Destruction

We have stressed that the lentiviruses are adapted to the very essence of the host immune system. Ironically, our attempts to understand HIV are teaching us more and more about the human immune system. Although we know that infection by HIV and SIV results in a progressive loss of T4 cells, that loss is not completely understood because, at any one time, only one in ten thousand to one in a million circulating T4 cells are infected with HIV.

Thus, the simple fact that HIV and SIV can destroy T4 cells through massive viral replication in vitro (Fig. 11) does not seem to explain the dramatic T4 cell depletion in vivo. Therefore, the search is on for other mechanisms. It has been discovered that antibodies and killer T8 lymphocytes in human AIDS patients are capable of attacking their own normal, uninfected T4 cells. This attack on self is probably due in part to molecular mimicry, that is, MHC II, which is expressed by normal activated T4 cells, may "look like" *gp*120 to anti-*gp*120 antibodies and to T8 killer cells. (As shown in Fig. 4, the T4 cell receptor CD4 binds to both MHC II and *gp*120.) These and other possible mechanisms of T4 cell depletion are listed in Table 4. The fact that we list them illustrates not only our knowledge but also our ignorance about how the depletion process works.

We do know that the loss of T4 cells from the body induces a progressive loss of immune regulation. Because T4 cells orchestrate directly or indirectly all of the other T, B, monocyte, and macrophage cells of the immune system

FUNCTIONS OF T4 HELPER CELLS

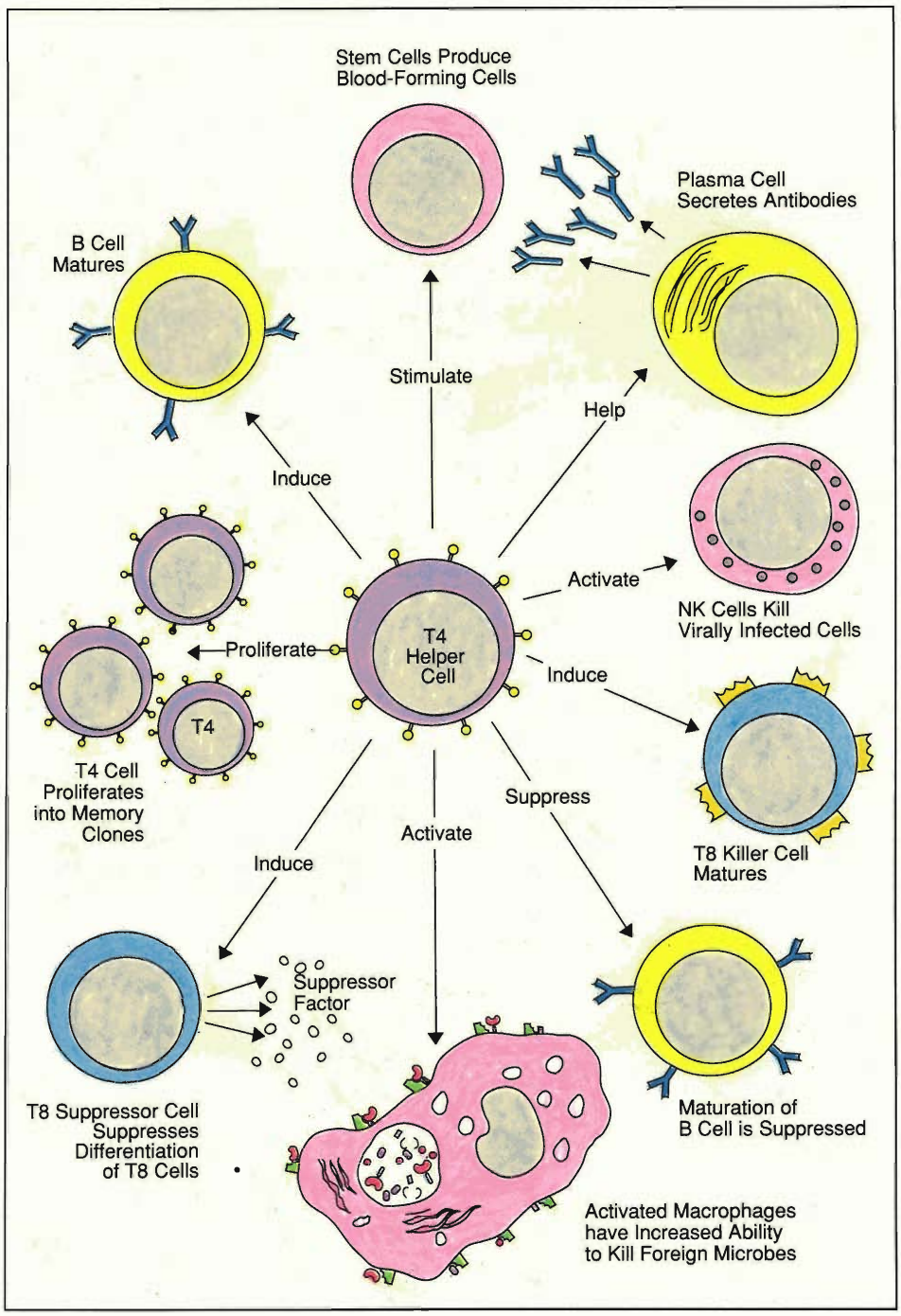
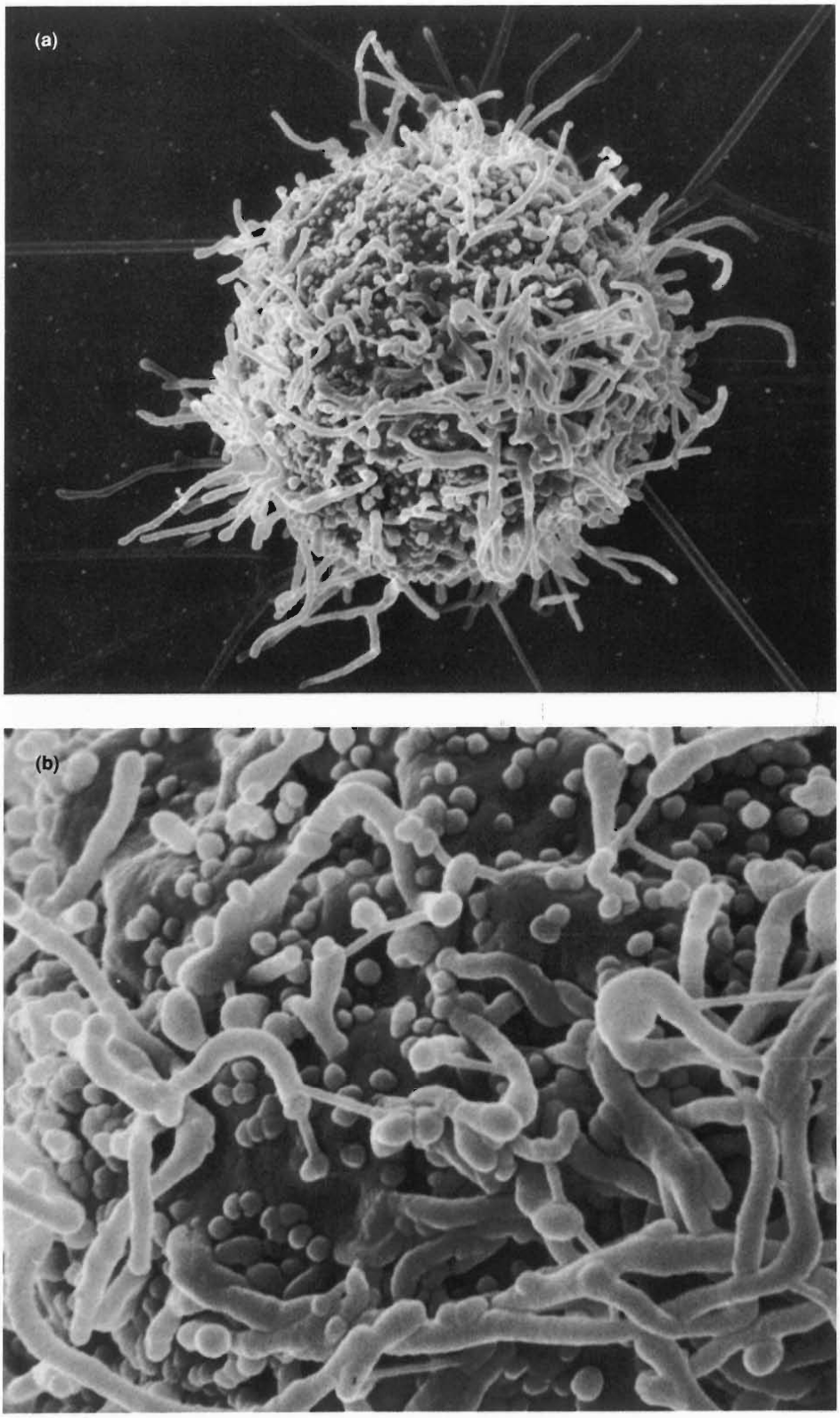


Fig. 12. T4 lymphocyte cells are called helper/inducer cells because they secrete many soluble chemicals that induce responses in other white cells. Thus T4 helper cells play a critical role in the immune response. The chemicals they secrete induce growth and differentiation of T and B lymphocytes, stimulate bone marrow stem cells, induce the killing function of T8 killer and natural killer cells, induce the suppression function of T8 suppressor cells, activate macrophages, and induce the functions of other nonlymphoid immune cells.

(Fig. 12), their loss leads to a general uncontrolled activation of the immune system, which is revealed by an excess of circulating antibodies in the blood and tissues. Moreover, helper events prompted by chemical messages from T4 lymphocytes are diminished or absent. In this setting of immune suppression, ubiquitous microbes in the environment, the body's own flora, and even some spontaneously formed tumor cells may flourish inappropriately.

HIV infection of monocytes, macrophages, and bone-marrow stem cells also leads to immune dysregulation. We have clear evidence that, in sheep and horses, respectively, the visna-maedi and EIA viruses infect monocytes and macrophages directly, although the specific celluar receptors involved have not yet been identified. Human macrophages become infected with HIV via CD4 or, perhaps, other receptors on the macrophage surface. Another infection mechanism is the binding and engulfment of antibody-coated HIV.

Whatever the mechanism of entry, the infection of monocytes and macrophages probably diminishes the performance of their various accessory functions, such as secretion of complement and clotting factors, tissue reorganization and repair, and killing of microbes and tumor cells. We have direct evidence from in vitro studies of visna-maedi of how the persistent infection of monocytes and macrophages stimulates the chronic activation of the immune responses shown in Fig. 5. In particular, lymphocytes in culture with visna-maedi-infected macrophages were found to produce a unique interferon, a soluble protein with three important effects. First, it retards monocyte maturation, thus indi-



VIRAL REPLICATION IN HUMAN T LYMPHOCYTES

Fig. 13. Scanning electron micrograph of HIV-infected human T4 lymphocyte. (a) A single cell infected with HIV showing virus particles and microvilli on the cell surface (magnified 7,000 times). (b) Enlargement of a portion of the cell surface in (a) showing multiple virus particles budding or attached to the cell surface (magnified 20,000 times). (Photograph courtesy of K. Nagashima and D. Chisholm, Program Resources, Inc., NCI-Frederick Cancer Research Facility.)

rectly slowing the rate of viral replication. Second, it restricts the rate of virus maturation, and last, but most important, it induces an unusually high expression of MHC II antigens and viral antigens on the surface of the macrophages. It seems that the high expression of MHC antigens, when presented in association with lentiviral antigens, chronically stimulates the series of immune reactions shown in Fig. 5, which subsequently leads to abnormal accumulations of virally susceptible host immune cells and, in some cases, causes local tissue destruction.

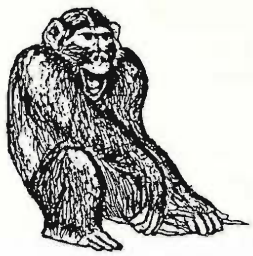
Inherited Host Responses?

How do some hosts prevent the types of immune dysregulation just described? Are there natural immune responses induced by lentiviruses that can lead to a state of protection? In attempts to develop a vaccine against lentiviral disease, we and other researchers have investigated all known immune responses that might lead to such a state. (Some of those studies are described in "The Search for Protective Host Responses.") The progress to date has been discouraging. So far, none of the responses have been found to be effective against lentiviral disease. Moreover, none of

them have yet been thoroughly studied and identified as the cause of protection in the virus-adapted species previously mentioned.

Thus, the natural history of the lentiviruses, as well as the current lack of success of vaccine studies, suggests that the carrier state found in some species is a direct effect of host adaptation. Such adaption probably involves a combination of traditional immune responses and accumulated changes in the host's immune-response genes and the virus's genes over long periods of time.

What are some of the possible inherited-adaptive properties that may confer the carrier state on the lentivirus-adapted host? (1) There may be an absence or only a limited number of cells in the adapted host that are pathologically susceptible to the viral infection, and those cells that are infected are not altered in their normal immune function. (2) Controlling genes inherent in the cell may limit the amount of viral replication to very low levels, allowing critical host immune cells to be replaced or controlled by the host immune system faster than they are compromised. (3) The virus may be regulated or prevented from flourishing by naturally inherited or nonspecifically acquired antiviral substances present in body fluids. These substances would include cross-reactive antibodies induced from other pathogenic agents or viruses, as well as various other species- associated antiviral substances in blood and serum. Approximately thirteen such substances have been described in the scientific literature, including a heat-stable lipoprotein that inhibits the visna-maedi virus of sheep. (4) The presence of nonpathogenic, host-adapted viruses may interfere with infection by lentiviruses or at least somehow limit uncontrolled lentiviral replication. (5) Infected cells of certain hosts may specifically produce and release soluble proteins during their viral infection that prevent the infection of cells nearby or at some other location in the body. Interferon is an example of this last possibility.



Lessons from the Chimpanzee, Man's Nearest Living Relative

Due to the disappointing search for a state of immune protection in HIV-infected humans and the fact that such humans appear to possess a complete, yet nonprotective, repertoire of antiviral immune responses toward HIV, we are currently looking at animal models of host adaptation. The only model for the human virus is the HIV I-infected chimpanzee. To date, approximately one hundred chimpanzees have been infected in various laboratories with different variants of HIV obtained from human AIDS patients and tissue cultures. Preliminary studies demonstrate a state of viral infection persisting for 4 to 5 years with no clinical manifestations of disease. It can be argued that 4 to 5 years is insufficient time for disease development, however, in humans infected for this period of time, multiple cellular and immunologic abnormalities are measurable. None of these immune destructive signs are found in the infected chimpanzees at any time to date. Although an even longer incubation period may be required before these animals show clinical signs, the results of our chimpanzee studies are provocative.

Chimpanzees have received HIV I from diverse sources in various laboratories, varying from HIV I-infected cells or cell-free virus derived in tissue culture to samples of whole blood, spleen, bone marrow, or thymus from humans with AIDS. Included in the list is the intracerebral administration of suspensions of brain tissue from patients dying of AIDS-associated pathology of the brain. Also, chimpanzees that were persistently infected with HIV I have been experimentally manipulated with immunosuppressing and immunostimulating protocols without developing AIDS-associated abnormalities.

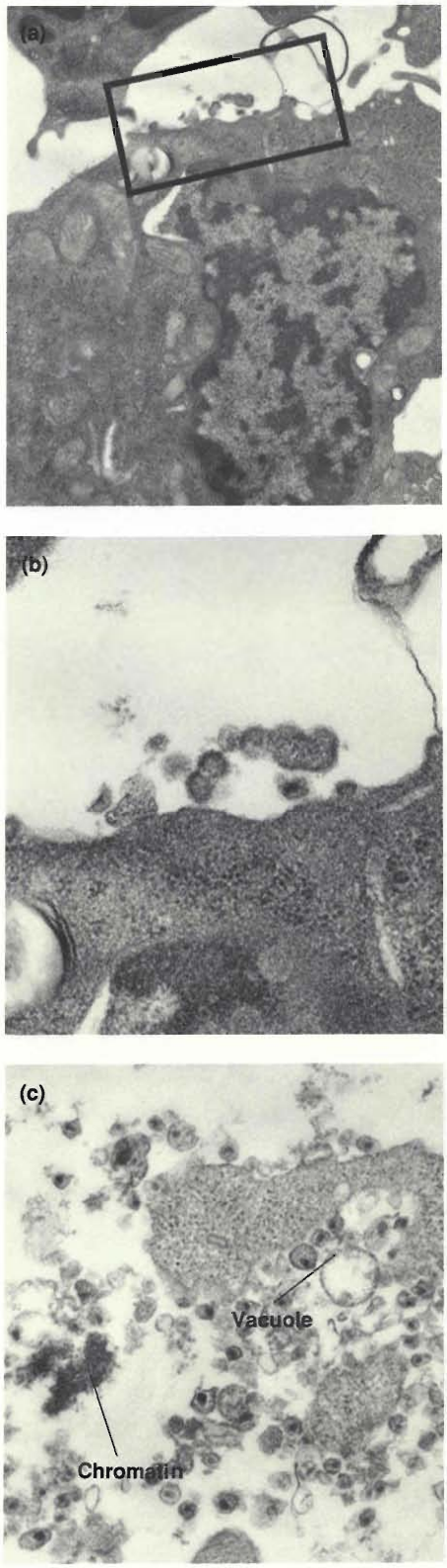
Four to six weeks after inoculating chimpanzees with as little as a single syncytial-forming unit of tissue-culture-derived HIV I, the virus can be reisolated by culturing lymphocytes from the blood of the infected animals. However, unlike the situation in HIV I-infected humans, cell-free virus could never be detected in the blood serum of chimpanzees at any time during the past 3 years. Two weeks after reisolation of the virus becomes possible, the infected animals make detectable antibodies to the viral antigens. In fact, the animals make antibodies that recognize all the known viral proteins recognized by antibodies from HIV I-infected humans. The animals also initially develop a virus-specific neutralizing antibody. With time, this neutralizing antibody is capable of neutralizing other HIV I variants, as happens in humans infected with HIV I. No abnormalities of the T4 or other lymphocytes are detected in the chimpanzee's immune system during this persistent infection.

In addition, no significant changes have been reported in the ratio of T4 to T8 lymphocytes in infected chimpanzees. During the infection the animals also make HIV I-specific cytotoxic T8 lymphocytes capable of killing HIV I-infected cells. More interesting, although humans infected with HIV I make autoreactive T8 lymphocytes capable of killing their normal cells, thus leading to immune dysregulation, chimpanzees do not. This further supports the thesis that the immune system of the

chimpanzee remains functionally intact during the persistent HIV I infection.

Studies following in vitro infection with HIV I show that all normal T4 lymphocytes from the blood of uninfected chimpanzees are capable of controlling the replication of the virus so that only small numbers of complete infectious virus particles are produced at any one time in these cells (Fig. 13). Also, the viral infection does not appear to kill the lymphocytes. More important, the monocytes and macrophages derived from the blood- and bone-marrow stem cells of experimentally inoculated animals do not appear to be readily infected by the virus. In support of this in vivo observation is the fact that current attempts to infect cultured chimpanzee monocytes and macrophages with human monocyte HIV I variants have failed. One other interesting finding is the naturally larger concentration of circulating T8 cells in the blood of chimpanzees. That population of cells in uninfected chimpanzees may be responsible for the antiviral controlling effect against HIV I. In contrast, in vitro studies of lymphocytes from the blood of HIV-infected humans and SIV-infected monkeys show that the presence of T8 lymphocytes seems to slow down but does not eliminate viral replication in the other lymphocytes.

The fundamental differences in the chimpanzee's response to HIV infection are being actively explored for application to the prevention and treatment of the human condition. Studies of the other SIV-adapted African primates mentioned previously should also contain clues to the various factors responsible for the carrier state present in these animals. As described in the section on SIV, the African green monkey and various other African primates appear to be successfully adapted to their virus, whereas the same virus when put into Asian primates leads to AIDS. The HIV I-infected chimpanzees also appear to be successfully adapted to HIV I. That fact suggests that an ancestral HIV I-like virus should be present in wild chimpanzee populations in central Africa. Furthermore, it looks like HIV I-infected humans are the counterpart to the SIV- infected Asian monkeys.



CONTROLLED VIRAL REPLICATION IN CHIMP LYMPHOCYTES

Fig. 14. Transmission electron micrographs of an HIV I-infected lymphocyte from a chimpanzee. The cell was taken from a culture that had been infected for 5 days and was at maximum virus production. (a) Only the boxed area of the cell membrane of this peripheral blood lymphocyte could be found budding HIV I particles (magnified 3500 times). (b) The boxed area in (a) at a magnification of 10,000. (c) To contrast with the chimp lymphocyte, we show a completely degenerated portion of an HIV-infected human lymphocyte magnified 10,000 times. Note the remaining portions of nuclear chromatin and cytoplasmic vacuoles as well as the presence of numerous viral particles.

Prospects for an AIDS Vaccine

Given our current understanding of lentiviral infections, it appears that conventional vaccine strategies are unsuitable for direct application to the prevention of lentiviral diseases. Since the time of Jenner and Pasteur, all successful human and animal antiviral vaccines have been made either from virus attenuated in tissue cultures (called modified-"live" virus particles), or from a part or parts (termed subunit) of the virus that activates the immune system. The vaccines confer immunity by eliciting what is termed an *anamnestic response*, which basically means to "not forget." On subsequent introduction of wild-type virus (other than lentiviruses) into the body, infection occurs, but the immune system, previously sensitized through vaccination, rapidly responds and elim-

inates the viral invader. The success of these vaccines was due primarily to the nature of the viruses involved. Viruses successfully blocked by vaccine-induced protection generally do not integrate themselves into the host's genome and do not exclusively parasitize the immune system of the body. A prototype for a subunit retroviral-based vaccine has been developed for cats against feline leukemia virus infection. However, further work is still required to optimize its usefulness and application to the prevention of lentiviral diseases. Recently, studies with a formalin-fixed Type D retrovirus were found to completely protect primates from a simian Type D retrovirus. As yet no vaccines against any of the retroviruses have been made from approaches using modified-live virus.

The immune activation induced by a successful vaccine is controlled by the immune system's activator and suppressor networks. With time, the induced protective state falls to levels of nonprotection, but the state is permanently programmed into the immune system's memory network. Reinfection of the host cells with the real pathogenic viral agent causes release of chemical signals that rapidly recruit and deploy the appropriate memory cells. These cells produce antibodies, T4 cells, and T8 cells that eliminate the virus before it can spread to a critical number of susceptible target cells and cause significant life-threatening disease.

A key point with regard to AIDS is the fact that the immune response generated by our current viral-based vaccines does not always prevent the initial infection. Since HIV is capable of integrating itself into the genetic material of infected cells, a vaccine would have to produce a constant state of immune protection, which could totally block the initial infection of the host cells at all times. Such complete and constant protection has never before been accomplished and works in direct opposition to the normal immune suppressor network, which dampens, or turns down, specific immune responses. A state of perpetual immune activation may have as yet undefined detrimental consequences to the host. Only through extensive and creative research will we be able to design the type of new generation vaccines required to defeat the AIDS virus and its distant relatives.

Analysis of the comparative spectrum of lentiviral diseases of animals and man are providing important clues to pathogenesis and host response. The nonsusceptible versus susceptible states that occur, respectively, in adapted versus nonadapted species need to be studied further. For lentiviral diseases it appears that protective immunity will not be conferred through the classical immune mechanisms but rather will require a state similar to that of host adaptation. The mechanisms of host adaptation has never been investigated nor understood in detail. Now the investigation of such mechanisms seems urgent. We will have to understand novel viral-controlling mechanisms, the nature of the immune state that prevents the initial infection, and methods for establishing persistent but nondeleterious states of protective immune activation. As yet, no antiviral vaccine can claim such protection. Nevertheless, continued studies of all lentiviruses, as well as other primate retroviruses, will surely reveal important clues to the understanding and control of the disease process in the animal kingdom. ■

Table 5

Problems with Vaccine Development against HIV

1. Integration of HIV genetic material into cellular DNA.
2. Regulatory genes responsible for controlled, low-level viral expression.
3. Similar cell populations serve as both targets of viral infection and vehicles of immune protection.
4. Viral activation and spread from antigen-presenting macrophages to T cells during normal immunogenic episodes.
5. Molecular mimicry between viral envelope proteins and MHC II molecules.
6. Rapid rate of envelope mutations.
7. Hypervariability of a single immunodominant neutralization epitope may act as a decoy to antibody producing cells.
8. Viral envelope proteins are poor immunogens due to high carbohydrate content.
9. Rapid shedding of viral envelope glycoproteins.
10. Cell-to-cell fusion, resulting in transmission of viral RNA without complete assembly of virus particles.
11. Presence of antibody induces viral latency.
12. Absence of complement-mediated cytolysis or direct complement activation.
13. No efficacious vaccine developed for any lentiviral infections of other species.
14. The need to maintain a constant level of protective immune activation in the face of an immune system suppressor network.

The Search for Protective Host Responses

How does a host usually develop a state of protection against an invading virus? Three major host responses to invading viruses include activation of complement, production of neutralizing and complement-fixing antibodies, and cell-mediated immune responses. Traditionally, when a new viral disease is recognized in a species, efforts to understand the protective immune states are derived from its surviving members. These individuals serve as immune benchmarks, and subsequent studies often reveal important clues to the eventual production of a vaccine. Here we will review studies of the major antiviral immune responses to HIV and see that none of them are completely effective, although some avenues of developing traditional vaccines for AIDS are still open.

Complement. One possible response to HIV would be the activation of the complement system, known to be a powerful, continuous, ever-present, microbe-eliminating system (see Fig. 11 in the main article). Complement is a group of serum proteins circulating in the bloodstream that bind to, become activated, and destroy invading microbes by creating holes in their surface membranes. Complement proteins are synthesized by activated macrophages, liver cells, and epithelial cells. Complement inactivates some Type C oncoviruses directly due to the presence of as yet undefined receptors on the viral envelopes. Complement can also work in conjunction with antibodies. The antibodies produced in response to a viral infection may bind both to complement and to the virus or virally infected cells, resulting in destruction of the intruder. The destruction of virally infected cells through this mechanism is called antibody-dependent, complement-mediated cytolysis (ACC). The lentiviruses as a group, unfortunately, appear to be resistant to destruction by complement.

Studies performed in my laboratory in 1987 showed no evidence of ACC activity in humans at various stages of AIDS despite the presence of large amounts of antibody directed against both HIV and HIV-infected cells. The absence of ACC is also documented in visna-maedi. No ACC activity has been reported for the other animal-lentivirus systems mentioned here. Both my lab and others have shown that human complement is incapable of inactivating HIV either directly or in the presence of neutralizing antibody. Recently, we have discovered a heat-sensitive serum factor in various laboratory and wild animal species that does inactivate the human AIDS virus in vitro. Further studies are underway to characterize this factor, or factors, and to understand how to recruit its activity in humans and why human complement does not work against HIV.

Neutralizing Antibody. Neutralizing antibodies have been shown to be one of the major lines of defense in viral diseases of human and other animals. Following the infection of the host by the AIDS virus, plasma cells produce antibodies directed against various parts of the virus. The antibodies are of two major types, functional (when they bind to the virus they inactivate or destroy it) and nonfunctional. A nonfunctional antibody recognizes various parts of the virus; however, they do not mediate any antiviral effects in vitro or in vivo. Also the nonfunctional antibodies can coat the virus and thereby block or interfere with otherwise effective antibodies, such as neutralizing or complement-fixing ones. An antibody that is coating a virus can also, as previously described, facilitate entry of the virus into monocytes and macrophages, thus infecting these cells. Some evidence for this type of antibody-facilitated infectiv-

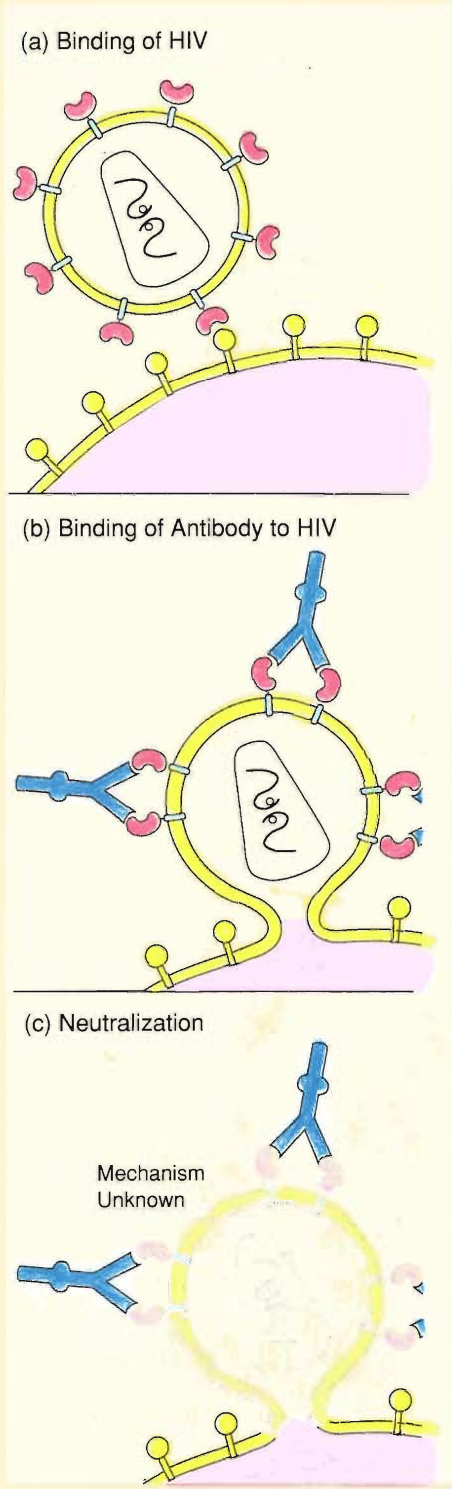
ity has been reported in the visna-maedi system.

Functional neutralizing antibodies are produced by plasma cells derived from B lymphocytes that have been specifically activated against a particular antigen. We have already indicated that the ability of the neutralizing antibody to inactivate lentiviruses is highly variable. Horses infected with the EIA virus make antibody that is capable of neutralizing the initial infecting viral strain. However, antigenic drift eventually produces viruses that can avoid those neutralizing antibodies.

Our information on visna-maedi is more detailed. In vitro, antibody against visna-maedi is capable of neutralizing the virus so that it cannot infect a cell. However, neutralization of the virus occurs only if the virus and antibody are allowed to interact for 15 minutes or longer before being introduced to the target cells. It appears that after this preincubation the antibody prevents the virus from attaching to the sheep's cells. However, when the virus and antibody are added to the target cells simultaneously, no neutralization of the virus occurs. These studies suggest that the antibody produced during the infection is not biologically functional in vivo. In the host the virus probably encounters and infects target cells before neutralizing antibody has sufficient time to neutralize it. The virus's escape from antibodies appears to be related to the high sugar content of the viral envelope proteins, which conceal neutralization epitopes (protein shapes that serve as antibody binding sites).

Fortunately, the neutralizing antibody present in HIV-infected humans, HIV-infected chimpanzees, and animals that have been vaccinated with the viral envelope protein *gp*120 are more effective. Recent detailed kinetic studies in my laboratory revealed that the serum from these hosts rapidly neutralizes the virus. Subsequent infectivity studies with HIV I demonstrated that the virus can be neutralized at various times, even after it has attached via the CD4 receptor to the host cell (Fig. 1). It appears that the virus binds to susceptible lymphocytes at the diffusion-limited rate of 4.0×10^{-9}M (see "The Kinetics of Viral Infectivity"). After binding, however, the virus only slowly enters the cell by the fusion process. Thus, neutralizing antibody is capable of neutralizing the virus during the 30 to 60 minutes between binding and entry into the lymphocyte. This is a singularly encouraging finding for vaccine development. However, only the sera from HIV-infected humans or HIV-infected chimpanzees were capable of neutralizing more than one HIV I strain. Moreover, these strains may only be a *sub*population of the virus present in any one infected individual. Studies of the role of neutralizing antibody in preventing infection of monocytes and macrophages will have to await the development of new assay methods.

Our studies also show that neutralizing antibody derived from sera of goats infected with the purified envelope of one HIV strain is also capable of neutralizing the virus either before or after it has bound to a target cell. The major limiting feature however was the narrow specificity of the neutralizing antibody produced. We, in collaboration with Jaap Goudsmit, Scott Putney, and others, have discovered that neutralizing antibody reacts only with the immunodominant neutralizing epitope of *gp*120 shown in Fig. 2. Further, this portion of *gp*120, which is about 30 amino acids in length, appears to be changing its amino acid content rapidly in infected humans and more slowly in chimpanzees. In particular, even the first viruses isolated from chimps infected with a specific and well-characterized human AIDS virus were resistant to a typing sera made



NEUTRALIZATION OF HIV

Fig. 1. In vitro studies suggest that neutralizing antibody against HIV can neutralize the virus even after it has bound to a target-cell membrane. The figure shows neutralizing antibodies attaching to the viral envelope after the virus has bound to and begun to fuse with the cell membrane. The antibodies somehow prevent infection, but the details of the neutralization mechanism are unknown.

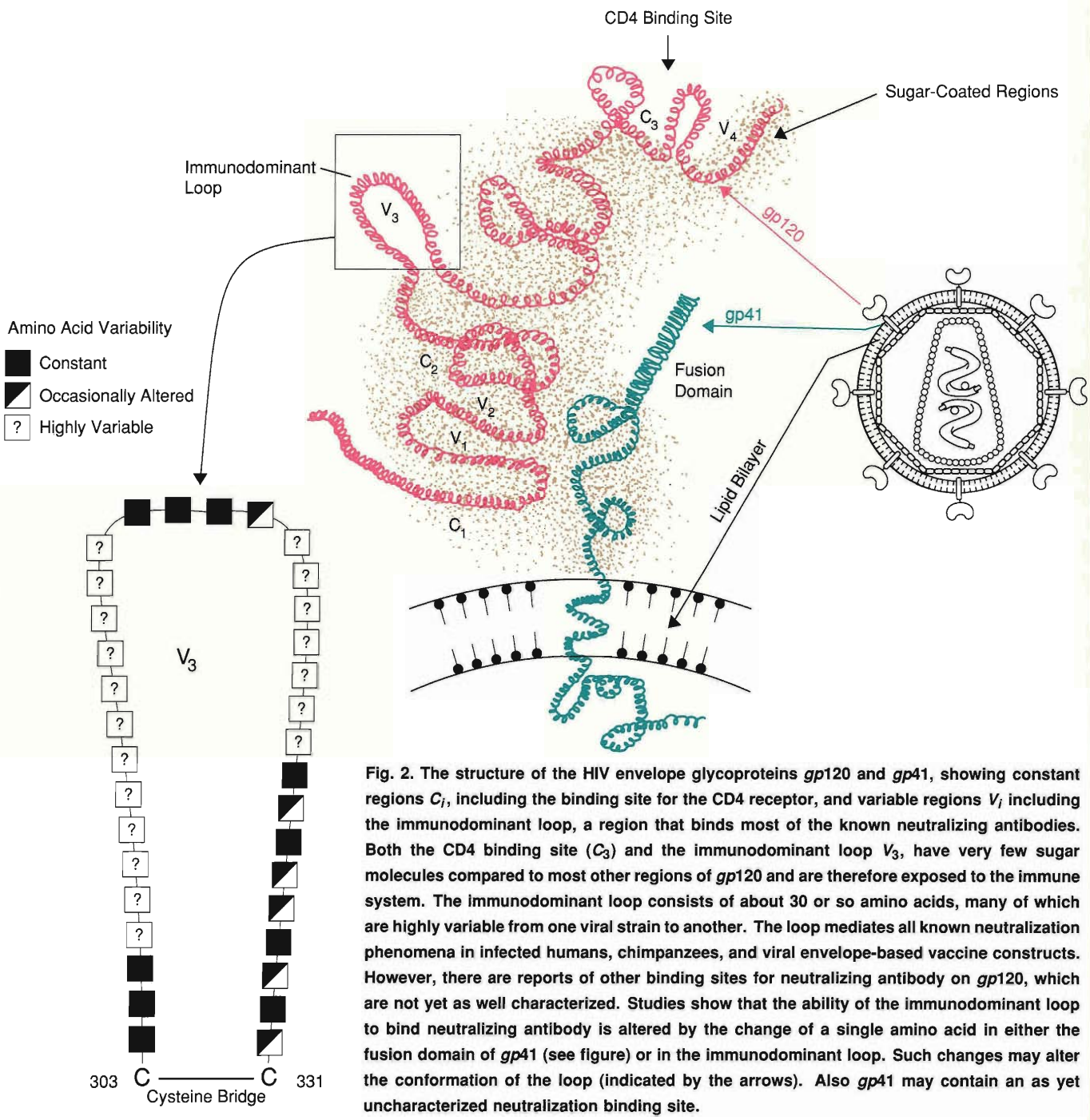


Fig. 2. The structure of the HIV envelope glycoproteins *gp*120 and *gp*41, showing constant regions C_i, including the binding site for the CD4 receptor, and variable regions V_i including the immunodominant loop, a region that binds most of the known neutralizing antibodies. Both the CD4 binding site (C_3) and the immunodominant loop V_3, have very few sugar molecules compared to most other regions of *gp*120 and are therefore exposed to the immune system. The immunodominant loop consists of about 30 or so amino acids, many of which are highly variable from one viral strain to another. The loop mediates all known neutralization phenomena in infected humans, chimpanzees, and viral envelope-based vaccine constructs. However, there are reports of other binding sites for neutralizing antibody on *gp*120, which are not yet as well characterized. Studies show that the ability of the immunodominant loop to bind neutralizing antibody is altered by the change of a single amino acid in either the fusion domain of *gp*41 (see figure) or in the immunodominant loop. Such changes may alter the conformation of the loop (indicated by the arrows). Also *gp*41 may contain an as yet uncharacterized neutralization binding site.

from goats immunized with *gp*120 of the original innoculated virus as well as sera from other chimps infected with the original virus. We are currently studying the amino acid sequence of the relevant pieces of the viral envelope protein in an effort to identify the location and the types of changes that occur during viral replication. Additional collaborative studies in our lab now indicate that other sites in the viral envelope also must contribute to the interaction between the neutralizing antibody and the immunodominant loop (see Fig. 2). When completed, the molecular study of the viral-envelopes from chimp isolates will provide a map of the mutation sites and allow for a better understanding of its complexity. Perhaps we will be able to identify a limited number of locations and variations that cover the spectrum of *gp*120 variations made during viral replication. We might then be able to manufacture an anti-*gp*120 vaccine that would be effective against all these variations.

Cell-mediated Immunity. We have just discussed the ineffectiveness of both complement and neutralizing an-

tibody in preventing infection by cell-free HIV particles. Finally, we turn to cell-mediated responses. As mentioned earlier, T8 killer cells are designed to destroy infected cells and are activated by T4 helper cells. The activation occurs when the T4 cells recognize an MHC-lentiviral antigen pair on the surface of infected macrophages and lymphocytes. The T8 cells then circulate around the body and kill any cells of the body displaying both the MHC and viral proteins. K, or killer cells (a subset of lymphocytes), and certain T cells can also destroy virally infected cells that do *not* present MHC antigens on their surface. One such mechanism, called antibody-dependent cell-mediated cytotoxicity, is the capacity of various antiviral antibodies to bind to the infected cells and thus direct the viral-killing K cells to them (see Fig. 10 in the main article).

Most HIV-infected humans display all these antiviral immune mechanisms and still progress to disease and death. One clue to their ineffectiveness may be the discovery that parts of the envelope of the feline leukemia virus, a member of the oncoviral subfamily, seem to suppress these antiviral immune strategies, thus adding to the persistence of the virus in the cat's body. Some reports suggest that the envelope of HIV may have a similar effect on the human immune system. Thus we have one possible explanation for the ineffectiveness of cell-mediated immune mechanisms against HIV. However, there have been no reports of other similar immunosuppressive effects for the other lentiviral infections of animals.

This short review of protective immune responses suggests that protection against HIV, if it can be developed, will probably have to involve various undefined elements of host-virus adaptive responses in addition to the known antiviral immune responses. ■

Further Reading

Scientific American October 1988. This entire issue is devoted to articles on AIDS, and each article has a valuable reading list of its own.

The Control of Human Retrovirus Gene Expression, edited by B. Robert Franza, Jr., Bryan R. Cullen, and Flossie Wong-Stall. 1988. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Timothy B. Crawford, William P. Cheevers, Paula Klevjer-Anderson, and Travis C. McGuire. 1978. Equine infectious anemia: virion characteristics, virus-cell interaction, and host responses. In *Persistent Viruses: ICN-UCLA Symposia on Molecular and Cellular Biology, Volume XI, 1978*, edited by Jack G. Stevens, George J. Todaro, and C. Fred Fox, pp. 727–749. New York: Academic Press.

Alfred S. Evans. 1989. Does HIV cause AIDS? An historical perspective. *Journal of Acquired Immune Deficiency Syndromes* 2:107–113.

Anthony S. Fauci. 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science* 239:617–622.

Matthew A. Gonda. 1986. The natural history of AIDS. *Natural History* 95:4.

Ashley T. Haase. 1986. Pathogenesis of lentivirus infections. *Nature* 322:130–136.

William A. Haseltine, Ernest F. Terwilliger, Craig A. Rosen, and Joseph G. Sodroski. 1988. Structure and function of human pathogenic retroviruses. In *Retrovirus Biology: An Emerging Role in Human Diseases*, edited by Robert C. Gallo and Flossie Wong-Stall. New York: Marcel Dekker, Inc.

Peter L. Nara, W. Gerard Robey, Larry O. Arthur, Matthew A. Gonda, David M. Asher, R. Yanagihara, Clarence J. Gibbs, Jr., D. Carleton Gajdusek, and Peter J. Fischinger. 1987. Simultaneous isolation of simian foamy virus and HTLV-III/LAV from chimpanzee lymphocytes following HTLV-II or LAV inoculation. *Archives of Virology* 92:183–186.

Peter L. Nara, W. Gerard Robey, Matthew A. Gonda, Stephen G. Carter, and Peter J. Fischinger. 1987. Absence of cytotoxic antibody to human immunodeficiency virus-infected cells in humans and its induction in animals after infection or immunization with purified envelope glycoprotein gp120. *Proceedings of the National Academy of Sciences* 84:3797–3801.

Peter L. Nara, William G. Robey, Larry O. Arthur, David M. Asher, Axel V. Wolff, Clarence J. Gibbs, Jr., D. Carleton Gajdusek, and Peter J. Fischinger. 1987. Persistent infection of chimpanzees with human immunodeficiency virus: serological responses and properties of reisolated viruses. *Journal of Virology* 61:3173–3180.

P. L. Nara, W. C. Hatch, N. M. Dunlop, W. G. Robey, and P. J. Fischinger. 1987. Simple, rapid, quantitative micro-syncytium forming assay for the detection of neutralizing antibody against infectious HTLV-III/LAV. *AIDS Research and Human Retrovirus* 3:283–302.

P. L. Nara and P. J. Fischinger. 1988. Quantitative infectivity microassay for HIV-1 and -2. *Nature* 332:469–470.

Peter L. Nara. 1989. HIV-1 neutralization: evidence for rapid, binding/postbinding neutralization from infected humans, chimpanzees, and gp120-vaccinated animals. In *Vaccines 89: Modern Approaches to New Vaccines Including Prevention of AIDS*, edited by Richard A. Lerner, Harold Ginsberg, Robert M. Channock, and Fred Brown, pp. 137–144. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

P. L. Nara, W. Hatch, J. Kessler, J. Kelliher, S. Carter, J. Ward, D. Looney, G. Ehrlich, H. Gendleman, and R. C. Gallo. 1989. The biology of HIV-IIIB infection in the chimpanzee: in vivo and in vitro correlations. *Journal of Medical Primatology*, in press.

O. Narayan, M. C. Zink, D. Huso, D. Sheffer, S. Crane, S. Kennedy-Stoskopf, P. E. Jolly, and J. E. Clements. 1988. Lentiviruses of animals are biological models of the human immunodeficiency viruses. *Microbial Pathogenesis* 5:149–157.

G. Pétursson, P. A. Pálsson, and G. Georgsson. 1989. Maedi-visna in sheep: host-virus interactions and utilization as a model. *Intervirology* 30 (supplement 1):36–44.

Howard M. Temin. 1989. Is HIV unique or merely different? *Journal of Acquired Immune Deficiency Syndromes* 2:1–9.

Peter Nara received a B.S. in zoology from Colorado State University in 1977 and, by 1986, had received an M.S., a D.V.M. (Doctor of Veterinary Medicine), and a Ph.D. from Ohio State University. For his doctoral work he studied mechanisms of viral inactivation, including identification of the responsible proteins, species distribution, and developmental biology, associated with a particular oncoviral inactivating factor. He is currently chief of the Virus Biology Section, Laboratory of Tumor Cell Biology, at the National Cancer Institute-Frederick Cancer Research Facility in Frederick, Maryland and is a member of numerous scientific committees that deal with AIDS, HIV, and related viruses. Recently, he completed a four-year comparative pathology residency at the Armed Forces Institute of Pathology. His major areas of research interest include comparative pathobiology, comparative tumor biology, and comparative virology—especially virology that will shed light on the troublesome retroviruses.

Acknowledgments

The author wishes to acknowledge the veterinary and medical researchers who have, over the past 40 years, studied these persistent viral infections of animals and have contributed to the knowledge that has rapidly advanced the research of human AIDS: Bjorn Sigurdsson, Gudmundur Georgsson, Halldor Thormar, Pall Palsson, Opendra Narayan, Ashley Haase, Neal Nathanson, Gudmundur Petursson, Travis McGuire, Lane Perryman, Y. Kono, H. Sentsui, Leroy Coggins, and Timothy Crawford. I would also like to extend my deepest personal regard to Necia Cooper, Dixie McDonald, and Gloria Sharp, editor, office manager, and graphics designer, as well as the rest of the Los Alamos Science staff, for their excellent technical and creative support so critical in the production of a good review article. I would also like to thank my courageous wife, Brenda, and my children, DeAnna and Kelly Ann, for their encouragement and support in these endeavors. Finally, I would like to thank my staff at the Virus Biology Section for their dynamic and enthusiastic support.

The editor and author would like to thank the authors, designer, and publisher of *Immunology* (by Ivan Roitt, Jonathan Brostoff, and David Male, Grove Medical Publishing, Ltd., London, 1985) for the inspiration their beautifully illustrated volume provided in the creation of this article.